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Abstract

(57) [Abstract]

(There is an amendment.)

[Problems to be Solved by the Invention]

This invention is to establish gene introduction method for skeletal muscle of dystrophin gene.

[Means to Solve the Problems]

As for this invention, hinge 1, hinge 4 of dystrophin gene and rod repeat structure of rod domain of at least one. Containing gene introduction medium for genetic therapeutic of the muscular dystrophy which consists of therapeutic agent, adeno attendance virus (AAV) vector or the wrench viral vector of muscular dystrophy which consists of these genes for the treatment of muscular dystrophy, which possesses base sequence, which hybridize it can do in base sequence or its salt basic arrangement being a length below 4.5 kb, the adeno attendance virus (AAV) vector, wrench viral vector, or adenoviridae vector which become. It is related to the therapeutic agent of muscular dystrophy, which consists of this said adenoviridae.

Claims

[Claim(s)]

[Claim 1]

At least one it possesses hinge 1, hinge 4 of dystrophin gene and rod repeat structure of the rod domain, in base sequence, or its salt basic arrangement which is a length of 4.5 kb or less hybridize gene for

treatment of muscular dystrophy which possesses base sequence which it can do.

[Claim 2]

The gene, which is stated in Claim 1, possesses over 2 rod repeat structure of rod domain.

[Claim 3]

Furthermore, the gene, which is stated in Claim 1 or 2, which possesses cysteine rich domain.

[Claim 4]

The gene, which is stated in any of Claim 1 ~ 3, which furthermore possesses act in binding domain.

[Claim 5]

The gene, which is stated in any of Claim 1 ~ 4, which furthermore possesses C terminal domain.

[Claim 6]

In base sequence or this said base sequence where gene is stated in Sequence Number 1 of sequence table hybridize gene, which is stated in any of the Claim 1~5, which possesses a base sequence.

[Claim 7]

Gene Sequence Number 3, 5 or 7 of sequence table or in base sequence or this said base sequence that can be hybridized, which is stated in any of the Claim 1~5, which possesses base sequence.

[Claim 8]

Gene Sequence Number 2, 4, 6 or 8 of sequence table or in base sequence or this said base sequence which amino acid sequence which can be hybridized, which is stated in any of Claim 1~7, which possesses base sequence.

[Claim 9]

Sequence Number 9 of sequence table or in base sequence or this said base sequence which is stated in Sequence

Number 11 hybridize gene, which possesses base sequence which it can do.

[Claim 10]

Therapeutic drug of muscular dystrophy, which consists of gene which is stated in any of Claim 1~8.

[Claim 11]

Gene introduction medium for genetic therapy of muscular dystrophy, which consists of adeno attendance virus (AAV) vector or wrench viral vector.

[Claim 12]

Containing gene which is stated in any of Claim 1~8, the gene introduction medium, which it states in Claim 7, which it becomes.

[Claim 13]

The vector containing gene, which is stated in any of Claim 1~8, which it becomes.

[Claim 14]

Vector adeno attendance virus (AAV) vector, adenoviridae vector or a vector, which is stated in Claim 13, which is a wrench viral vector.

[Claim 15]

Containing vector, which is stated in Claim 13 or 14, therapeutic drug of the muscular dystrophy, which it becomes.

Specification

[Description of the Invention]

[0001]

[Technological Field of Invention]

As for this invention, hinge 1, hinge 4 of dystrophin gene and rod repeat structure of rod domain at least one possessing its salt basic arrangement being a length below 4.5 kb. Containing gene introduction medium aforementioned gene for genetic therapy of the muscular dystrophy which consists of therapeutic agent

adeno attendance virus (AAV) vector or the wrench viral vector of muscular dystrophy, which consists of these gene for the treatment of muscular dystrophy, which possesses base sequence which hybridize it can do in base sequence, the adeno attendance virus (AAV) vector, wrench viral vector or adenoviridae vector which it becomes. And it regards therapeutic agent of muscular dystrophy, which consists of these vector.

[0002]

[Prior Art]

With genetic muscle disorder of serious illness, which takes heredity form of X chromosome linkage characteristic recessive, furthermore with (1 out of 3,500 new born males) Duchenne type muscular dystrophy (DMD), Author Emery, A.E.H. (1993) Duchenne Muscular Dystrophy, 2nd ed., Oxford University Press, Oxford. Cf., where pathopoiesis frequency is high, the dystrophin gene (14 kb), which is a cause gene as result of positional cloning to be isolated. [Koenig, M., Hoffman, E. P., Bertelson, C. J., Monaco, A. P., Feener, C. and Kunkel, L. M., (1987) Cell (0092 - 8674), Vol. 50, page 509 - 517]), concerning relation of gene fault between the disease, including the participation of dystrophin connection protein, research is advanced.

[0003]

But, furthermore as for 1/3 of pathopoiesis people, in egg cell level of maternal mutation, to which with skeletal muscle of DMD infant patient for dystrophin, which defect has been done. [Zubrzycka-Gaarn, E. E., Bulman, D. E., Karpati, G., Burghes, A. H. M., Belfall, B., Klamut, H. J., Talb ot, J., Hodges, R. S., Ray, P. N. and Worton, R. G. (1988) Nature (London) (0028 - 0836) 333, 466 - 469] and others.

[Arahata, K., Ishiura, S., Ishiguro, T., Tsukahara, T., Suhara, Y., Eguchi, C., Ishihara T., Nonaka, I., Ozawa, E. and Sugita H. (1988) Nature (London) Vol. 333, page 861 - 863.]. It is difficult with cytoskeleton protein, which it is related to membrane, to expect to the drug treatment, for the sake of, prenatal diagnosis is not effective always.

[0004]

Therefore, genetic therapy is considered.

In order to establish genetic therapy for muscular dystrophy, efficiency to be high method where safe region is wide is desired in relation to the skeletal muscle.

So far, research which uses adenoviridae vector whose infection power is strong was done actively, [Ragot, T., Vincent, N., Chafey, P., Vigne, E., Gilgenkrantz, H., Couton, D., Cartaud, J., Briand, P., Kaplan, J.- C., Perricaudet, M. and Kahn, A. (1993) Nature (London) Vol. 361, page 647 - 650], [Vincent, N., Ragot, T., Gilgenkrantz, H., Couton, D., Chafey, P., Gregoire, A., Briand, P., Kaplan, J.- C., Kahn, A. and Perricaudet, M. (1993) Nature Genet. Vol. 5, page 130-134], [Deconinck, N., Ragot, T., Marfichal, G., Perricaudet, M. and Gillis, J.M. (1996). Proceedings of the National Academy of Sciences of the United States of America Vol. 93, page 3570 - 3574], and [Acsadi, G., Lochmiiller, H., Jani, A., Huard, 1., Massie, B., Prescott, S., Simoneau, M., Petrof, B.J. and Karpati, G. (1996) Hum. Gene Ther. Vol.7, page 129-140].

[0005]

But, as for adenoviridae vector of first generation, length of introducible gene is limited by 7.5 kb introduced gene is not taken in to chromosome. antigenicity of vector had held problem that is high, [Acsadi, G., Lochmiiller, H., Jani,

A., Huard, l., Massie, B., Prescott, S., Simoneau, M., Petrof, B.J. and Karpati,G. (1996) Hum. Gene Ther. Vol. 7, page 129-140].

[0006]

Divides dystrophin molecule to domain of 4 of act in binding domain rod domain cysteine rich domain and C terminal domain is possible densely from structural N terminal, [Koenig, M., Monaco, A. P. and Kunkel, L. M. (1988) Cell Vol. 53, page 219 - 228].

[0007]

Among these, 3 domain which exclude rod domain are domain which is necessary in order to connect plasmalemma and act in filament, [Hemmings, L., Kuhlman, P. A. and Critchley, D. R. (1992) Journal of Cell Biology Vol. 116, page 1369 - 1380], and [Suzuki, A., Yoshida M., Hayashi, K., Mizuno, Y., Hagiwara, Y. and Ozawa, E. (1994) European Journal of Biochemistry Vol. 220, page 283 - 292].

[0008]

Rod domain (It consists of repeat and hinge structure of 24.) occupied 76% of dystrophin molecule, from fact that homology of spectrin is high, relation with lining structure of membrane was expected, but gene deficiency of this domain is assumed that Becker type muscular dystrophy (BMD) where disease is light in clinical is caused [Beggs, A. H., Hoffman, E. P., Snyder, J. R., Arahata, K., Specht, L., Shapiro, F., Angelini, C., Sugita, H. and Kunkel, L. M. (1991) American Journal of Human Genetics, Vol. 49 and page 54 - 67].

Actually, approximately 60% of rod domain it was deficient, BMD patient of extremely mild disease is reported. [England, S. B., Nicholson, L. V. B., Johnson, M. A., Forrest, S. M., Love, D. R., Zubrzycka-Gaarn, E. E., Bulman, D. E., Harris, J. B. and Davies, K. E. (1990) Nature Vol. 343,

page 180 - 182].

[0009]

With appearance of this kind of patient as opportunity, mini-dystrophin gene of 6.3 kb which are deficient cloning is done 60% of the rod domain, introduces into mdx mouse as transformer gene, or adenovirus vector of the first generation installs in one and when it introduces into mdx mouse skeletal muscle, finding of muscular dystrophy is improved is proven densely, [Ragot, T., Vincent, N., Chafey, P., Vigne, E., Gilgenkrantz, H., Couton, D., Cartaud, J., Briand, P., Kaplan, J.- C., Perricaudet, M. and Kahn, A. (1993) Nature Vol. 361, page 647 - 650], [Vincent, N., Ragot, T., Gilgenkrantz, H., Couton, D., Chafey, P., Gregoire, A., Briand, P., Kaplan, J.- C., Kahn, A. and Perricaudet, M. (1993) Nature Genet Vol. 5, page 130-134], [Deconinck, N., Ragot, T., Marfichal, G., Perricaudet, M. and Gillis, J.M. (1996) Proceedings of the National Academy of Sciences of the United States of America Vol. 93, page 3570-3574], and [Acsadi, G., Lochmiiller, H., Jani, A., Huard, 1., Massie, B., Prescott, S., Simoneau, M., Petrof, B. J. and Karpati, G. (1996) Hum. Gene Ther. Vol. 7, page 129-140].

[0010]

Research is advanced with direction of two concerning length restriction of gene which mini- dystrophin gene and antigenicity of the vector which combination of adenoviridae vector of first generation has held and, it installs and is possible densely.

It is a development of adenoviridae vector (gut-less adenovirus vector) of new generation where the one removed all adenoviridae a protein gene.

This method antigenicity of vector is lightened not only, made there arrangement of gene whose 35 kb or

less is long possible, [Kochanek, S., Clemens, P. R., Mitani, K., Chen, H.-H., Chan, S. and Caskey, C. T. (1996) Proceedings of the National Academy of Sciences of the United States of America Vol. 93, page 5731 - 5736].

But, helper virus which is necessary for producing vector mixes to also final product, with present state, the fact that lacZ gene is required as marker for measuring potency it remains as problem.

[0011]

Direction of another is development of new viral vector whose antigen residence is lower.

Recently, adeno attendance virus (AAV) vector was developed as the vector where gene introduction which long period stabilizes with installation to chromosome skeletal muscle is possible, furthermore anti-genicity is low, made densely clear, [Fisher, K. J., Jooss, K., Alston, J., Yang, Y., Haecker, S. E., High, K., Pathak, R., Raper, S. E. and Wilson, J. M. (1997) Nature Med. Vol. 3, page 306-312].

But problem when is that introduced gene is restricted to only 4.5 kb this vector combining with dystrophin gene [Ferrari, F. K., Xiao, X., McCarty, D. and Samulski, R. J. (1997) Nature Med. Vol. 3, page 1295-1297].

[0012]

[Problems to be Solved by the Invention]

This invention, overcoming these problem, is to establish gene introduction method for the skeletal muscle of dystrophin gene.

[0013]

These inventors, in order to obtain functional dystrophin gene of applicable minimum size even in theother viral vector, rod portion of mini-dystrophin gene furthermore constructed the dystrophin gene of

shortening type, which is deficient.

Next, installing dystrophin gene of shortening type in adenoviridae vector, it introduces into skeletal muscle of culture skeletal muscle cell and maturity mdx mouse verification it did whether or not revelation of dystrophin connection protein (DAP), which has been connected with stability and dystrophin of the revelation recovers.

[0014]

In addition, it introduces these inventors, adenoviridae vector which rearranges lacZ gene, culture skeletal muscle cell and maturity mouse skeletal muscle, CAG promoter [Niwa, H., Yamamura, K. and Miyazaki, J. (1991) Gene Vol. 108, page 193 - 200] brings revelation of highest gene, immune reaction for adenoviridae protein and introduced gene product attendant upon introduction of adenoviridae, is caused, but those it differs depending upon strain of mouse it made densely clear.

From these results, it applies to genetic therapeutic directly, as for adenoviridae vector of first generation which holds many problem, that it is superior as gene introduction method for cultured cell and maturity mouse skeletal muscle, you thought, you had decided to use as expression assay of shortening type dystrophin gene.

[0015]

[Means to Solve the Problems]

Hinge 1, hinge 4 of dystrophin gene and rod repeat structure of rod domain at least one it possesses this invention, it regards gene for treatment of muscular dystrophy which possesses base sequence which hybridize it can do in base sequence, or its salt basic arrangement which is a length of 4.5 kb or less.

Rod repeat structure of rod domain 2 or more it is possible to have possessed the gene of this invention.

Furthermore, gene of this invention regards gene, which furthermore, is possible to have possessed cysteine rich domain, act in binding domain and/or C terminal domain.

In addition, this invention regards therapeutic agent of muscular dystrophy, which consists of these gene.

[0016]

In addition, this invention consists of adeno attendance virus (AAV) vector, it regards gene introduction medium for genetic therapeutic of muscular dystrophy.

Namely, this invention uses adeno attendance virus (AAV) vector where the anti-genicity is little as gene introduction medium for genetic therapeutic of muscular dystrophy, densely it is something which is made one of feature.

This invention, adeno attendance virus (AAV) vector, before containing the any of gene of this invention which was inscribed, regards gene introduction medium for genetic therapeutic of muscular dystrophy which becomes.

[0017]

Furthermore, this invention, before containing any of gene of this invention which was inscribed, vector, preferably adeno attendance virus (AAV) vector, adenoviridae vector which becomes, or regards wrench viral vector.

In addition, this invention before regards also therapeutic agent of muscular dystrophy which consists of vector which was inscribed.

[0018]

AAV vector has several benefit concerning gene introduction for

skeletal muscle, but in order to overcome problem of length restriction (4.6 kb) of the introduced gene, furthermore it is necessary to have dystrophin gene which has function with miniature.

Mini- dystrophin gene (6.3 kb) which is used in past research exceeds limit of introduction largely.

Then, with length which it installs in AAV vector and is possible densely, construction of dystrophin gene of effective minimum limit was supposed in the treatment.

[0019]

Total length type dystrophin gene, code has done act in binding domain, rod domain, cysteine rich domain, and C terminal domain from N terminal.

These inventors constructed rod shortening type dystrophin cDNA of 6 kinds which furthermore shorten the rod domain with human mini-dystrophin gene (6.3 kb) which has 8 rod repeat as material (A of Figure 1).

All structure has left act in binding domain, cysteine rich domain, and C terminal domain of N terminal.

[0020]

The ΔDysAX2, AX11, AH3 and M3 which design are done, respectively have both of rod repeat and hinge 1 and hinge 4 of 3, 3, 2 and 1.

In shortening type dystrophin of these 4, in order with fusion portion to maintain cell structure [Koenig, M. and Kunkel, L.M. (1990) Journal of Biological Chemistry Vol. 265, page 4560 - 4566.] to presumption triple of rod repeat, cDNA design was done (B of Figure 1).

On one hand, as for ΔDysH1 or H4, as for rod repeat it does not have completely, respectively, hinge 1 has which of 4 (A of Figure 1, C of Figure 1).

Base sequence of primer and

oligonucleotide which are used for constructing of these cDNA is shown in Table 1 of Working Example 1 which it mentions later.

[0021]

N terminal which these inventors constructed, includes hinge 1 and the C terminal which includes hinge 4 are kept, shortening type dystrophin gene ΔDysM3 where just one has rod repeat, function which improves phenotype of muscular dystrophy with introduction experiment to newborn mdx mouse skeletal muscle, has been verified densely.

In comparison with the ΔDysM3 namely, rod domain all is lacked in structural concerning small dystrophin, but localized it does in the plasmalemma as dystrophin concerning dystrophin gene which keeps hinge 1 and the hinge 4, but it cannot improve finding of muscular dystrophy.

In addition, miniature dystrophin Dp71 from C- terminal finding of muscular dystrophy has been known also that it deteriorates rather from last half of hinge 4.

Therefore, so far, as for the ΔDysM3, it is thought that it is a minimum dystrophin functional unit.

[0022]

Next, you express concerning construction of these dystrophin gene.

Namely, inserting NotI/ SalI fragment of gene of 6.3 kb which are a human mini- dystrophin cDNA, in NotI/SalI site of plasmid pBluescriptII (SK+) (Stratagene Corp. supplied), it produced the plasmid pBSBMD.

[0023]

Plasmid pBSBMD and primer F1/R1 which it acquires (Table 1 reference) or after cutting off the PCR fragment which amplifying is done, with AflII/ XhoI, it inserted in the AflII/ XhoI

site of pBSBMD with F2/ R2 (Table 1 reference), respectively, produced the pBSΔDysAX2 or pBSΔDysAX11.

Next, after cutting off PCR product which amplifying is done with the MunI/ Hind III, it is inserted in MunI/ Hind III site of pBSBMD with pBSBMD and the primer F4/ R4 (Table 1 reference) of template, produced pBSΔDysM3.

Consequently, fragment which is produced with earning ring of oligonucleotide F3/ R3 (Table 1 reference), was used for connection of AflII/ Hind III site of the pBSBMD, pBSΔ DysAH3 was produced.

Occasion where it connects, in order to maintain triple helical structure of the rod repeat, design it did these inserted fragment.

Amino acid sequence of rod repeat which it connects is shown in B of the Figure 1.

[0024]

As a result, the ΔDysAX2, AX11, AH3 and M3 keep act in binding domain cysteine rich domain and C terminal domain of N terminal, furthermore respectively have both of rod repeat and hinge 1 and 4 of 3, 3, 2 and 1.

It produced the ΔDysH1 and plasmid of 2 it has cDNA of the ΔDysH4, from pBS ΔDysM3 (A of Figure 1).

In order to exclude EcoO109I site of 1, it cut off pBS ΔDysM3 with ApaI, after smoothing, self ligation did, produced pBSΔ DysM3b.

Using pBSΔDysM3 and primer F5/R5 (Table 1 reference) of template, after cutting off PCR product which amplifying is done with EcoT22I/EcoO109I, it inserted this in EcoT22I/EcoO109Isite of pBSΔ DysM3b, produced pBSΔ DysH1.

[0025]

For producing pBSΔ DysH4, pBSΔDysM3 was designated as template, primer

F5/ R6 (Table 1 reference) or F6/ R7 (Table 1 reference) was used and PCR reaction of 2 kinds was done separately.

Using primer F5/ R7 (Table 1 reference) with mixture of PCR product of 2 kinds which it acquires as template, it did PCR reaction of second.

After cutting off fragment which it acquires with EcoRV, this it inserted between EcoRV site of 2 in pBSΔ DysM3.

Amino acid sequence of junction region is shown in C of Figure 1.

As for the ΔDysH1 or H4 which it acquires, as for rod repeat it does not have completely, respectively, hinge 1 has which of 4 (A of Figure 1).

[0026]

Figure 1 is something which shows construction of shorteningtype dystrophin gene which has rod repeat of various numbers.

A of Figure 1 is human total length type dystrophin gene, mini-dystrophin gene and the list figure of shortening type dystrophin cDNA which is produced newly.

The ΔDysAX2, ΔDysAX, ΔDysAH3 and in order to construct the ΔDysM3, it cut off with restriction enzyme which shows rod domain of center of themini-dystrophin cDNA in right side of respective structure.

In order re-to construct rod repeat structure, using PCR amplifying fragment or synthetic DNA fragment, itconnected both ends which it acquires.

The ΔDysH1 and in order to construct the ΔDysH4, after cuttingoff, using PCR amplifying fragment with restriction enzyme which illustrates the ΔDysM3, itconnected both ends.

Dotted line shows junction.

Size of cDNA and estimated molecular

weight of shortening type dystrophin areshown in right side.

Act in binding domain with sporadically box, rod domain with box of the white-out (Respective repeat is shown with box of 1), cysteine rich domain it illustrates with box where slanted line enters, and C terminal domain with box which attaches shade.

Box of black shows hinge.

As for statement of hinge you followed description of the M.Koenig and L.M.Kunkel.

[0027]

As for B of Figure 1, the ΔDysAX2 (AX2), the ΔDysAX11 (AX11), the ΔDysAH3 (AH3) and reconstruction in the ΔDysM3 (M3) amino acid sequence of the rod repeat which is done is shown.

Vertical line shows junction rank.

Triangle and dotted line show gap in order alignment of rod repeat optimization to do and position of deficiency, (With M.Koenig and L.M.Kunkel).

CS1 and CS2 show consensus sequence of repeat of 24 of the dystrophin.

As for CS1, amino acid which among Beta vulgaris L. var. saccharifera Alef. (sugar beet) of 24 is found at least in 8 Beta vulgaris L. var. saccharifera Alef. (sugar beet), as for CS2 5, amino acid where is seen 6 or7 in Beta vulgaris L. var. saccharifera Alef. (sugar beet) is shown.

[0028]

As for C of Figure 1, the ΔDysH1 (H1) and with amino acid sequence ΔDysH1 (H1) of junction region in the ΔDysH4 (H4), you connect directly the hinge 1 to cysteine rich domain.

With the ΔDysH4 (H4), you connect directly act in binding domain to hinge 4.

Tyrosine (T) (star), which is hinge 1 with lineage of XLCM of North America mutation had made in alanine (A).

Dotted line under hinge 4 shows Wwdomain, among WWdomain, amino acid which most is retained is shown with underline.

[0029]

Next, you express concerning method, which introduces respectiveshortening type dystrophin cDNA, which is acquired with aforementioned method into adenoviridae vector.

With COS-TPC [Miyake, S., Makimura, M., Kanegae, Y., Harada, S., Sato, Y., Takamori, K., Tokuda, C. and Saito, I. (1996) Proceedings of the National Academy of Sciences of the United States of America Vol. 93, page 1320-1324]. Emonosubstituted type rearrangement adenoviridae vectorwhich reveals each shortening type dystrophin can be produced.

[0030]

Respective shortening type dystrophin cDNA, ΔDysAX2, AX11, AH3, M3, H1 and H4 which areacquired with aforementioned method, were inserted to in CAG revelation unit [Niwa, H., Yamamura, K. and Miyazaki, J. (1991) Gene Vol. 108, page 193 - 200] of cassette cosmid pA XCAwt [Kanegae, Y., Lee, G., Sato, Y., Tanaka, M., Nakai, M., Sakaki., T., Sugano, S. and Saito, I. (1995) Nucleic Acids Research Vol. 23, page 3816 - 3821].

This revelation unit shows strong revelation in vitro (literature of ibid others and in vivo) [Araki, K., Araki, M., Miyazaki, J. and Vassalli, P. (1995) Proceedings of the National Academy of Sciences of the United States of America Vol. 92, page 160 - 164], it is known densely.

[0031]

Each it rearranged and production of

adenoviridae was done by homology rearrangement between DNA terminal protein conjugate of cosmid and Ad5 dl x [Saito, I., Oya, Y., Yamamoto, K., Yuasa, T. and Shimojo, H. (1985) Journal of Virology Vol. 54, page 711 - 719] whichare acquired in 293 intracellular.

Rearrangement adenoviridae vector which it acquires, AxCA ΔDys it designated, with method [Kanegae, Y., Makimura, M. and Saito, I. (1994) Japanese Journal of Medical Science Biology Vol. 47, page 157-166] which was already expressed, it was multiplied, it was refined and it measured potency.

Each AxCA ΔDys in phosphate-buffered conversion raw food water (PBS) whichincludes 10% glycerol, -was retained with -80 deg C.

[0032]

You verified revelation of shortening type dystrophin in theculture skeletal muscle cell which uses rearrangement adenoviridae vector of this invention following way.

Namely, in order shortening type dystrophin is done and to be correctcopying translation to inspect densely, infection doing each AxCA ΔDys in mouse skeletal muscle cell stocks C2C12 cell, you analyzed Western plot.

Each it rearranged into C2C12 cell and infection did adenoviridae at ratioof 100 moi, it induced differentiation after that, with exchangeof fermentation broth.

After infection 3 days, cell it recovered.

It separated whole cell extract (20;mu g/lane) with SDS-PAGE (5% acrylamide), after copying, reactedwith monoclonal antibody DYS2 in PVDF membrane.

This antibody reacts to last 17 amino acid of dystrophin.

Result is shown in Figure 2.

As for lane 1 of Figure 2 with those from non- infection C2C12 cell, as for lane 2 being something which uses AxCA ΔDysAX2, as for the lane 3 being something which uses AxCA ΔDysAX11, as for lane 4 being something which uses AxCA ΔDysAH3, as for lane 5 beingsomething which uses AxCA ΔDysM3, as for lane 6 beingsomething which uses AxCA ΔDysH1, lane 7 is something which uses AxCA ΔDysH4.

MW in Figure 2 shows molecular weight (kDa).

[0033]

As for respective shortening type dystrophin gene, (Figure 2, lane 2~6) which shows the size, which is estimated, as for the ΔDysH4 appeared in largeposition in comparison with estimate (103 kDa) (Figure 2, lane 7).

As for product of AxCA ΔdysH4 (Figure 2, lane 7) mobility was slow it waspresumed with in comparison.

As for dystrophin of endogenic with culture skeletal muscle cell it did notdetect.

Because because, cell was not differentiated in muscle tube cell which matures in fully.

When quantity of shortening type dystrophin is compared, the ΔDysM3 showed highest expression level.

As for these results, AxCA Δdys, which is rearranged in the effective infection did in culture skeletal muscle cell, shortening type dystrophin isrevealed under controlling CAGpromoter, furthermore, the ΔDysM3 protein stabilizing, most reveals showed densely.

[0034]

Furthermore, it rearranges and, it introduced AxCA ΔDys whichis rearranged in order to inspect whether or not shortening type

dystrophin of this invention which uses adenoviridae vector, with muscle fiber reveals in stability in in vivo, into front tibia muscle (TA) of maturity mdx mouse directly, analyzed immunity histological (Figure 3, photograph which is substituted to drawing).

Rearrangement adenoviridae, was introduced into front tibia muscle ofmaturity mdx mouse directly.

Vector quantity, which it introduces is quantity which is statedin Table 2 of Working Example 4 which it mentions later.

7 days later of injection, it removed TA from mouse, used freeze fracture and rabbit polyclonal antibody anti-C and dyed dystrophin antibody.

This antibody recognizes C terminal of dystrophin.

[0035]

As for B10 of Figure 3 with normal maturity C57BL/10 mouse, as for mdx of Figure 3 with non- introduction mdx mouse, as for AX2 of Figure 3 with AxCA ΔDysAX 2, as for AX11 of Figure 3 with AxCA ΔDysAX11, as for AH3 of Figure 3 with AxCA ΔDysAH3, as for M3 of Figure 3 with AxCA ΔDysM3, as for H1 of Figure 3 with AxCA ΔDysH1, H4 of Figure 3 has shown case where AxCA ΔDysH4 isused respectively.

bar in photograph, we have shown scale, length of the bar is 100;mu m, it has shown densely.

[0036]

In order to be reported already, with HE dyeing it rearranged and necrosis of invasion and muscle fiber where mononuclear cell is strongwith adenoviridae was detected.

Dystrophin positive fiber forming crowd in periphery of domain, which receives damage had tendency, which appears.

All shortening type dystrophin which

excludes the ΔDysH1, whenexamining on same slide of one layer even, it revealed strongly in plasmalemma in comparison with dystrophin of C57BL/10 mouse in normal control.

As for ratio of dystrophin positive fiber, it was many clearly in comparison with the revertant fiber, which is seen in mdx skeletal muscle.

Furthermore, dystrophin positive fiber is not rivertant fiber making use of P23a antibody [Yoshida, M. and Ozawa, E. (1990) Journal of Biochemistry Vol. 108, page 748 - 752] for rod repeat of 19th of dystrophin, you verified densely.

[0037]

Strength of immunostaining of dystrophin, however it had changed largelybetween fiber, strong immunofluorescence being consistent in skeletal muscle which introduces AxCA ΔDysM3, was observed in skeletal muscle whichintroduces AxCA ΔDys, (Figure 3).

In contrastive, signal of dystrophin intestinal characteristic fiber with plasmalemma was very weak and discontinuous regarding skeletal muscle whichintroduces AxCA ΔDysH1.

[0038]

In order to appraise effect of each shortening type dystrophin in the skeletal muscle of mdx mouse, domain of 3 place which formed cluster from skeletal muscle which introduces respective AxCA ΔDys pick up itdid these inventors, it appraised quantity of of line careless theshortening type dystrophin is revealed and strength of immunofluorescence of the dystrophin, separately.

Result, was summarized to Table 2.

As for these results, to effective localized is possible theshortening type dystrophin which has both of rod domain and hinge 1 and 4 it is short,

to plasmalemma, it has suggested densely.

As seen in the ΔDysH1, deficiency of hinge 4 became resultwhich decreases localized to plasmalemma largely.

[0039]

Next, it examined concerning revelation recovery of dystrophin connection protein (DAP) in plasmalemma.

In order to appraise function of dystrophin as key molecule inorder to form dystrophin-DAP conjugate, as for these inventors, AxCA ΔDys revelation of DAPs in plasmalemma of mdx skeletal muscle after introducingwas inspected.

In order to look at recovery of dystrophin connection protein in the plasmalemma of mdx skeletal muscle which AxCA ΔDysM3 injection is done, itintroduced gene with method which is explained with Figure 3 and it dyed antibody.

Result is shown in Figure 4 (photograph which is substituted to drawing).

Muscle fiber, which reveals dystrophin in mdx mouse, which introduces AxCA ΔDysM3, β-dystroglycan, α- salcoglycan, and for α1-cyntlophine was strongly dyed with antibody.

With dystrophin negative fiber (star in Figure 4), as for DAP it was a negative.

With mdx skeletal muscle which AxCA ΔDysH1 injection is done, as for the signal of dystrophin positive fiber with plasmalemma it was weak extremely.

With that kind of fiber, it did not detect DAP in plasmalemma.

Bar in photograph, we have shown scale, length of the bar is 50;mu m, it has shown densely.

[0040]

With mdx skeletal muscle, with skeletal muscle which introduces AxCA ΔDys other than AxCA ΔDysH1 [Ohlendieck, K. and Cam pbell, K.P. (1991) Journal of Cell Biology Vol. 115, page 1685 - 1694] (Figure 4) revelation of DAPs having decreased, revelation with plasmalemma of DAPs, recoveredconsiderably in dystrophin positive fiber.

Strength of immunofluorescence of DAPs resembled interest deepespecially, regardless of expression level of dystrophin.

But, with mdx skeletal muscle which introduces AxCA ΔDysH1, as for the immunofluorescence of DAPs, which parallels to plasmalemma it was detection difficult.

Especially, with dystrophin positive fiber of mdxs keletal muscle, which introduces AxCA ΔDysH1, the β -dystroglycan and signal of α -salcoglycan was low extremely.

From these results, as for shortening type dystrophin, which is revealed with plasmalemma other than the ΔDysH1 revelation of DAPs of the plasmalemma recovers understood densely in effective.

[0041]

With cannot introduce to maturity mouse skeletal muscle of these rearrangement adenoviridae vectors, with antigenicity of viral vector, revelation of long period of gene introduction product is expected densely.

But, because with gene introduction to newborn mouse, tolerance isformed, whether or not it introduces to newborn mdx mouse skeletal muscle, concerninginside AxCA ΔDysM3 of rearrangement adenoviridae vector which installs shortening type dystrophin gene, it improves phenotype of muscular dystrophy long period by revealing, verification it

did.

[0042]

AxCA ΔDysM3 and mixture 6 ;mu l of AxCALacZ were introduceddirectly in 腓 fore-edge muscle center of mdx mouse one side hind limb of 1 week afterraw.

4 weeks later, it removed skeletal muscle of 腓 fore-edge muscle section of hind limb, H&E dyed, X-Ga l it dyed and it dyed dystrophin.

As a result, when adenoviridae in order to verify introduction of one, you dye X-Ga l concerning fore-edge muscle group of the hind limb side which filled adenoviridae vector, most it could recognize the fiber which is introduced gene into high rate, among fore-edge muscle groups shallow in finger flexor (flexor digitorums uperficialis).

When immunostaining of dystrophin was done concerning this β-Gal positive domain, the dystrophin had revealed in most fiber.

Concerning same portion, dyeing H&E, when in detail you observe, thenon-inlet side finger flexor (flexor digitorums uperficialis) with by comparison shallow, modified necrosis image of muscle and quantity of center nucleus fiber had decreased considerably.

[0043]

Whether or not with this invention shortening type dystrophin which the design is done, stabilizing in muscle cell rearrangement adenoviridae vector which installs shortening type dystrophin gene by infection doing in skeletal muscle of culture skeletal muscle cell stocks C2C12 and maturity mdx mouse, itreveals these inventors, as for result which is examined, adenoviridae which has wide infection limits vector in one and skeletal muscle thecase where it introduces into skeletal muscle of maturity mdx mouse due toespecially combining

strong CAG promoter, revelation of shortening type dystrophin is compared was possible densely.

[0044]

Rod repeat the ΔDysM3 which has only 1 showed highest revelation in the in vitro (in vitro).

Clay menth, etc. produced shortening type dystrophin (3.0, 4.4 and 5.7 kb deficiency) of 3 kinds which have dystrophin frame deficiency of rod domain [Clemens, P. R., Krause, T. L., Chan, S., Korb, K. E., Graham, F.L. and Caskey, C.T. (1995) Hum. Gene Ther. 6, 1477-1485].

These, 1 5 and 1 0 or have rod repeat of 6.

As for he and others, produced amount of these dystrophin, it is not something which is decided by only size of deficiency at the time of introduction experimenting for culture skeletal muscle cell, it showeddensely.

These inventors, in addition, unless it depends on quantity of rod, conclusion it did stability of shortening type dystrophin which hasdeficiency in rod domain.

As for these results, as for size of deficiency it agreed with finding which is seen in BMD patient that produced amount of dystrophin andis not related also which of weight of disease.

[0045]

Introducing AxCA ΔDys into skeletal muscle of maturity mdx mouse when and, the ΔDysM3 revealed in same way as shortening type dystrophin, which has many rod repeat in effective.

Frequency of muscle fiber which dystrophin has revealed had the tendency, which is proportionate to virus quantity which is prescribed.

In addition, it is not case that higher dimensional structure of

correct ΔDysM3 is decided. In order it is a stability regarding skeletal muscle of maturity mouse and to have participated densely, it is thought.

[0046]

Concerning AxCA ΔDysH1 and AxCA ΔDysH4, virus of the large amount was introduced into skeletal muscle of maturity mdx mouse in sameway as other AxCA ΔDys, but those revelations were low clearly in comparison with other ΔDys.

As for this, the ΔDysH1 and the ΔDysH4 have been deficient the rod repeat together completely, it probably is a cause densely.

Especially, hinge 4 revelation of the ΔDysH1, which is deficientwas low extremely.

In hinge 4 "WW domain," [Sudol, M., Bork, P., Einbond, A., Kastury, K., Druck, T., Negrini, M., Huebner, K. and Lehman, D. (1995) Journal of Biological Chemistry (0021 - 9258, JBCHA3). 270 and 1473 3 - 14741.] are included, that this domain the β-dystroglycan to XPPXY motif of , dystrophin molecule is sustained to plasmalemma, it is lectured, [Einbond, A. and Sudol, M. (1996) FEBS Letters 384, 1- 8]. With that, these inventors, the β- dystroglycan because connection to can decreases, presumed the ΔDysH1 that destabilization it did.)

[0047]

The ΔDysH4 has been deficient hinge 1.

Importance of hinge 1 domain was pointed out recently.

In lineage of X chromosome linkage characteristic dilated cardiomyopathy of North America, the miss sense mutation identification is done in hinge 1 domain, structure of dystrophin molecule haschanged, it was supposed densely [Ortiz-Lopez, R., Li, H., Su, J., Goytia, V. and

Towbin, J.A. (1997) Circulation 95 and 243 4 - 2440].

From this kind of reason, that might, you thought in decrease ofrevelation of the ΔDysH4 defect of hinge 1 having participated.

[0048]

In order to be estimated from research [Wells, D. J., Wells, K. E., ASante, E. A., Turner, G., Sunada, Y., Campbell, K. P., Walsh, F. S. and Dickson, G. (1995) Hum. Mol. Genet. 4, 1245 - 1250] of transgenic of the mini- dystrophin cDNA, if, domain of C terminal side is kept even with small shortening type dystrophin like the ΔDysM3, DAPs is accumulated was possible densely in mdx mouse skeletal muscle.

Due to experiment of namely, this invention, as for shortening type dystrophin which has both of hinge 4 and cysteine rich domain, accumulates DAPs to plasmalemma was proven to effective densely.

But, fact that it should observing means is not a meaning where recovery of DAPs always means prevention or reduction of the pathopoiesis of muscular dystrophy.

[0049]

DAPs recovering in plasmalemma, there are times when it is an insufficient in improvement of dystrophin function.

With one of molecular type of dystrophin, Dp71 gene which lacks the actin binding domain of rod domain and N terminal with experiment which itintroduces as transformer gene mdx mouse, DAPs showed complete recovery of, as for effective improvement was not in phenotype of the muscular dystrophy in spite, [Cox, G. A., Sunada, Y., Campbell, K. P. and Chamberlain, J. S. (1994) Nature Genet. 8, 333 - 339], and [Greenberg, D. S., Sunada, Y., Campbell, K. P., Yaffe, D. and Nudel, U. (1994) Nature

Genet. 8, 340 - 344].

[0050]

On one hand, Chamberlain and others, it constructs consecutiveshortening type dystrophin gene, mdx mouse, introducing as transformer gene when it examined, it has N terminus side to hinge 1 and C terminus side of hinge 4 or less, but stabilizing in membrane, it reveals dystrophin of type which rod portion all defect is done, but You cannot see improvement in phenotype of muscular dystrophy, densely it has made clear.

In order from this viewpoint, to prove function with in vivo of shortening type dystrophin ΔDysM3, experiment whose long term revelation is possible is necessary.

[0051]

Really, these inventors, introducing adenoviridae vector which the ΔDysM3 the code is done in mdx mouse skeletal muscle of newborn, 4 weeks later, when it examined effect, with portion where adenoviridae vector is introduced, has obtained result that center nucleus fiber which is an index of muscle regeneration which it occurs as muscle modified decrease and muscle modified result almost disappears.

Because you try, that in order to decide whether or not this shortening type dystrophin, improves phenotype of muscular dystrophy, expression system of long period probably is more necessary concerning this point furthermore experiment which uses transgenic mouse becomes necessary, might.

[0052]

Regarding to this invention, shortening type dystrophin which keeps the rod repeat at even 1, with skeletal muscle of mdx mouse which matures reveals showed densely in effective.

Already in order to be clear, adenoviridae vector of first generation causes the strong immune reaction in host.

[0053]

Then, regarding genetic therapeutic for muscular dystrophy, in future, immune reaction isnot induced in host, and, use of new kind of vector which gives long period revelation of introduced gene is examined.

Especially, adeno attendance virus (AAV) vector has benefit that canbe expected revelation which is stabilized due to fact that introduced gene is taken in to chromosome, in skeletal muscle.

[0054]

However, as for gene which can be inserted in this vector barely, it was limited to 4 - 4.5 kb.

Therefore, concerning dystrophin gene, as for gene of total length of 14 kb of course, to mini- dystrophin gene of 6.3 kb, as for inserting it is impossible.

The ΔDysM3 cDNA of 3.7 kb where only 1 keeps shortening type dystrophin gene especially rod repeat which is acquired with this invention is quite the satisfactory gene which is inserted in adeno attendance viral vector.

[0055]

As been clear from result above, it is something where hinge 1, hinge 4 of dystrophin gene and rod repeat structure of rod domain at least one it possesses gene for treatment of muscular dystrophy of this invention, possesses base sequence which the hybridize it can do in base sequence, or its salt basic arrangement which is a length of 4.5 kb or less and densely makes feature.

If gene of this invention, rod repeat structure of rod domain 1 it had been supposed to have possessed, but when

depending, 2 or more, preferably 2 or 3 it is possible to have possessed.

As for these rod repeat structure, those which completely possess same base sequence are desirable, but part of that with other base being substituted also furthermore other base sequence being added also in addition, the base of part had could have been deficient.

[0056]

As for gene of this invention, furthermore, those which have possessed cysteine rich domain, act in binding domain, and C terminal domain are desirable.

If as for cDAN of this invention, total length should have been 4.5 kb or less, below preferably 4.2 kb and below more preferably 4.0 kb, furthermore even below preferably 3.7 kb is good.

[0057]

Gene of this invention can use this as therapeutic agent of muscular dystrophy.

It can also use method which is used from until recently as the method which introduces gene of this invention into patient, but installing gene of this invention in adeno attendance virus (AAV) vector, it is desirable to use.

Introduction method can adopt known method.

[0058]

In addition, this invention is something which offers gene introduction medium for the genetic therapeutic of muscular dystrophy which consists of adeno attendance virus (AAV) vector.

Adeno attendance virus (AAV) vector was used as gene introduction medium for the genetic therapeutic of other disorder, but it is something where possibility which you can use with

this invention for first time as gene introduction medium for the genetic therapeutic of muscular dystrophy is ascertained, discovers new application of this said vector.

As for gene introduction medium for genetic therapeutic of muscular dystrophy, before containing the any of gene of this invention which was inscribed, those which become are desirable, but gene introduction medium for genetic therapeutic of muscular dystrophy of the this invention is not something which is limited in these.

[0059]

Adeno attendance virus (AAV) vector of this invention before is something which contains any of gene of this invention which was inscribed.

As for especially restriction it is not in method which introduces gene of this invention into adeno attendance virus (AAV) vector, it can introduce due to method which person skilled in the art usually does.

In addition, it can produce adenoviridae vector of this invention, by introducing into adenoviridae vector before any of gene of the this invention which was inscribed due to conventional method .

[0060]

You can use therapeutic agent of muscular dystrophy which consists of adenoviridae of the this invention, with conventional genetic therapeutic method and same method which use virus.

[0061]

[Working Example(s)]

Listing Working Example below, you explain this invention in detail, but this invention is not something which is limited in these Working Example.

[0062]

Working Example 1 (Construction of rod shortening type dystrophin gene)

Dystrophin gene which furthermore shortens rod domain making use of method which is shown below, 6 kinds was constructed (A reference of Figure 1).

As next, shown plasmid of 4 it has cDNA of shortening type dystrophin (ΔDys) which is named AX2, AX11, AH3, M3 below, it produced.

Base sequence of primer and oligonucleotide which are used for constructing cDNA, is shown in Table 1.

[0063]

[Table 1]

プライマー	プライマーの配列（5’－3’）	配列の位置
F1	GCCGGCGAACAACTTAAGGTATTG	1799-1816
2	GCCGGCCTTAAGGAGGTCAATACTGAG	8936-8950
3	TTAAGGTATTGAACACCAGATGGA	1806-1816, 9269-9281
4	GCCGGCCAATTGGGAAGTAAGCTG	1409-1426
5	GGAACATGCATTCAACATCGCC	796-817
6	CAGGAAGTGGAAGCCCACAGGGACTTTGGTCCAG	953-964, 9329-9350
R1	GCCGGCCTCGAGACTTGATAACATTTC	2005-1991
2	GGCGCCTTGACTTTCTCGAGGTGATC	9144-9125
3	AGCTTCCATCTGGTGTTCAATACC	9285-9269, 1816-1810
4	GCCGGCAAGCTTCCATCTTGAATTTAG	1501-1486
5	CGGCAGGGCCTTCTGCAGTCTTCGGTCTTCAGGAGCTTCC	9564-9545, 1189-1174
6	GTCCCTGTGGGCTTCCACTTCCTGGATGGC TTC	9340-9329, 964-944
7	ATCTGCAGGATATCCATGG	9657-9639

[0064]

Although it anneals directly for DNA fragment formation, you used DNA sequence oligonucleotide F3 and R3 of synthetic oligonucleotide which is used, for constructing the shortening type dystrophin in Table 1.

You used other oligonucleotide, as primer for PCR reaction.

Underline is suitable to base sequence (Gene (0378 - 1119, GENED6)

Bank accession number M18533) of human dystrophin cDNA.

After cutting off PCR fragment which amplifying is done with AflII/ XhoI, itinserted in AflII /XhoI site of pBSBMD with pBSBMD and primer F1 /R2 or the F2/ R2 of template, respectively, produced pBSΔ DysAX2 or the pBSΔ DysAX11.

After cutting off PCR product which amplifying is done with MunI/ HindIII, itinserted in MunI/ HindIIIsite of pBSBMD with pBSBMD and primer F4/ R4 of the template, produced pBSΔ DysM3.

Fragment which is produced with earning ring of oligonucleotide F3/ R3,was used for connection of AflII/ HindIII site of pBSBMD, pBSΔ DysAH3 was produced.

[0065]

On one hand, it produced the ΔDysH1 and plasmid of 2 it has the cDNA of the ΔDysH4, from pBSΔDysM3 (A reference of Figure 1).

First, in order one to exclude EcoO109I site, it cut off the pBSΔDysM3 with ApaI, after smoothing, self ligation did and made pBSΔ DysM3b.

Using pBSΔDysM3 and primer F5 /R5 of template, after cutting off PCR product which amplifying is done, with EcoT22I/ EcoO109I, it inserted in the EcoT22I/ EcoO109Isite of pBSΔ DysM3b, produced pBSΔ DysH1.

For producing pBSΔ DysH4, using primer F5/ R6 or F6/ R7, with pBSΔDysM3 as template, it did PCR reaction of 2 kinds, separately.

Using primer F5/ R7 with mixture of PCR product of 2 kinds which it acquires as template, it did PCR reaction of second.

After cutting off fragment which it acquires with EcoRV, this it inserted between EcoRVsite of 2 in pBSΔ DysM3.

Amino acid sequence of junction region is shown in B of Figure 1 and the C of Figure 1.

[0066]

M3, AX11, AX2, of 4 kinds which it acquires and base sequence of cDNA of AH3 Sequence Number 1, 3, 5, of sequence table and are shown respectively in 7.

In addition, H1 of 2 kinds and base sequence of cDNA of the H4 Sequence Number 9 of sequence table and are shown respectively in 11.

Amino acid sequence which code is done Sequence Number 2, 4, 6, 8, 10 of sequence table and, is shownrespectively in 12 with these cDNA .

[0067]

Working Example 2 (Production of rearrangement adenoviridae vector which reveals the shortening type dystrophin)

With COS-TPC method, Emono substituted type rearrangement adenoviridae vector which reveals each shortening type dystrophin was produced.

Respective shortening type dystrophin cDNA, ΔDysAX2, AX11, AH3, M3, H1 and H4, were inserted to in CAG revelation unit of cassette cosmid pA XCAwt.

This revelation unit shows strong revelation in vitro and in in vivo.

Each it rearranged and production of adenoviridae vector was done by the homology rearrangement between DNA terminal protein conjugate of cosmid and Ad5 dl x which are acquired in 293 intracellular.

Rearrangement adenoviridae vector which it acquires, AxCA ΔDys it designated, with method which is already expressed, it multiplied, it refined and it measured potency.

Each AxCA ΔDys in phosphate- buffered

conversion raw food water (PBS) which includes 10% glycerol, was retained with -80 deg C.

[0068]

Working Example 3 (adenoviridae vector gene introduction to culture skeletal muscle cell which uses)

It spread one [Yoshida, S., Fujisawa-Sehara, A., Taki, T., Arai, K. and Nabeshima, Y. (1996) Journal of Cell Biology 132,181 - 193] (Approximately 1.0 x10^5) of subclone of C2C12 myoblast, in 6 cm collagen coating dish, 1 day it cultured in DMEM which includes 20% (vol/vol) fetal calf serum.

In myoblast infection doing AxCA ΔDys at ratio of 100 plaques-forming unit/cell (pl aque-formin g unit (pfu) /cell (moi)), multiplication column area it replaced to differentiating culture medium which includes DMEM and 5% (vol/vol) equine blood plasma.

3 days later, cell it recovered, suspension did in SDS- PAGE dissolution buffer (15% SDS, 70 mM Tris-HCl pH 6. 8, 5% β-mercaptoethanol (β-mercatoethanol), 10 mM EDTA).

[0069]

Per 1 lane, it separated cell dissolved liquid of 20;mu g with 5% SDS- PAGE, the electro brobuing membrane (Imm obillon (TM), Millipore).

Dystrophin monoclonal antibody DYS2 which plot 100 times is diluted (Novocastra) with incubation wasdone.

This antibody recognizes last 17 amino acid of human dystrophin.

It detected rabbit anti-mouse IgG1 which immunity conjugate on plot, peroxidase labelling is done (Zymed) with making use of ECL Western blotting detection reagent

(Amersham).

[0070]

Result is shown in Figure 2.

The ΔDysH4 is excluded, respective shortening type dystrophin showed size which is estimated.

With comparison of amount of expression of shortening type dystrophin, the ΔDysM3 showed highest expression level.

As for these results, AxCA ΔDys which is rearranged in the effective infection does in culture skeletal muscle cell, shortening type dystrophin is revealed has shown densely under controlling CAGpromoter.

[0071]

Working Example 4 (using adenoviridae virus vector (in vivo) gene introduction to mouse skeletal muscle of mdx).

Before in vivo gene introduction, in order to remove glycerol, it passed through stock of each AxCA ΔDys to Chroma Spin™ column (Clontech) which is saturated with PBS.

AxCA ΔDysliquid 50; mu l which it refined, direct injection (intramuscular injection) in the front arriving at bone muscle of left foot of mdx mouse of 12 - 16 weeks old making use of syringe needle of 27 gauge.

Quantity and result of each vector which it introduces are shown in following Table 2.

[0072]

[Table 2]

組換アデノウイルス	ウイルスの投与量 (x 10^8pfu/ 筋)	ジストロフィン陽性繊維 平均（範囲）	形質膜における免疫蛍光の強度	n
AxΔDysAX2	8.6	32% (22 -39)	++	4
AxΔDysAX11	2.2	27% (11-56)	++	4
AxΔDysAH3	14	33% (15-45)	++	4
AxΔDysM3	16	33% (22-51)	+++	8
AxΔDysH1	6.0	12% (3-22)	+	3
AxΔDysH4	13	21% (16-31)	++	3

[0073]

Table 2 making use of amount used and adenoviridae vector of vector shortening type dystrophin cDNA case where it introduces to mdx skeletal muscle has shown result of quantitative analysis.

"" sign in Table 2 shows percent of dystrophin positive fiber of selective domain, "" sign signal strength with plasmalemma of dystrophin has shown the result which from 0 is appraised in +++.

1 week later, it was removed skeletal muscle, freezing it did in is opentane which was cooled with liquid nitrogen.

Gene introduction was done, and from C57BL/10 skeletal muscle of mdx skeletal muscle and normal control which gene introduction have not been done, preparing cutting of 6;mu m on slide of same one layer, air dry after doing, 10 min it locked with acetone.

[0074]

Immunohistological staining was done making use of antibody which is listed next.

Rabbit polyclonal antibody recognizing most C terminal 25 amino acid of dystrophin (It procured from anti-C、 Nonomura (Y.Nonomura, Dr.), rabbit polyclonal antibody which recognizes amino acid 2360 to 2409 of dystrophin which is suitable to rod

repeat of 19 th (It procured from P23a, Yoshida (M.Yoshida, Dr.) [Yoshida, M. and Ozawa, E. (1990) Journal of Biochemistry 108,748 - 752], the β- di goat polyclonal antibody, rabbit polyclonal antibody for α - mokey glycal (It procured from Wakayama (Y.Wakayama, Dr.) [Wakayama, Y., Inoue, M., Murahashi, M., Shibuya, S., Jimi, T., Kojima, H. and Oniki, H. (1996) Ann. Neurol. 39, 217-223], the rabbit polyclonal antibody α-1 syntlophine for amino acid 191 to 206 [Peters, M. F., Kramarcy, N. R., Sealock, R. and Froehner, S. C. (1994) NeuroReport 5, 1577 - 1580] (It procured from Kameya (S.Kameya, Dr.)

[0075]

It detected goat anti- rabbit IgG which primary antibody, FITC labelling is done (Tago Imm unologicals), or making use of rabbit anti- goat IgG (Organon Teknika).

Using laser scanning Confocal imaging system MRC-1000 (Bio-Rad), it recorded result.

[0076]

Result is shown in Figure 3.

As a result, to effective localized is possible shortening type dystrophin (ΔDysAX2, AX11, AH3 and M3) which has both of rod domain and hinge 1 and 4 it isshort, to plasmalemma it has suggested densely.

Defect arrow of hinge 4 which is seen in the ΔDysH1 became the result which decreases localized to plasmalemma largely.

[0077]

Working Example 5 (Revelation recovery of dystrophin connection protein in plasmalemma)

In order to appraise function of dystrophin as key molecule inorder to form dystrophin- DAP conjugate, AxCA ΔDys revelation of DAPs in plasmalemma of mdx skeletal muscle

after introducing was inspected.

With mdx skeletal muscle, with skeletal muscle which introduces AxCA ΔDys other than AxCA ΔDysH1 [Ohlendieck, K. and Campbell, K. P. (1991) Journal of Cell Biology 115, 1685 - 1694] (Figure 4 reference) revelation of DAPs having decreased, revelation with plasmalemma of DAPs, recovered considerably in dystrophin positive fiber.

[0078]

Working Example 6 in vivo gene introduction for newborn mdx mouse skeletal muscle)

In fore-edge muscle center of mdx mouse one side hind limb of 1 week after raw, the AxCA ΔDysM3 and mixture 6;mu 1 of AxCALacZ were introduced directly.

4 weeks later, it removed skeletal muscle of fore-edge muscle section of hind limb, H&E dyed, X-Ga 1 it dyed and it dyed dystrophin.

As a result, when adenoviridae vector in order to verify introduction of one, you dye X-Ga 1 concerning fore-edge muscle group of the hind limb side which filled adenoviridae, most it could recognize the fiber which is introduced gene into high rate, among fore-edge muscle groups shallow in finger flexor (flexor digitorums uperficialis).

When immuno- staining of dystrophin was done concerning this β- Gal positive domain, the dystrophin had revealed in most fiber.

Concerning same portion, dyeing H&E, when in detail you observe, the non-inlet side finger flexor (flexor digitorum superficialis) with by comparison shallow, modified necrosis image of muscle and quantity of center nucleus fiber had decreased considerably.

[0079]

[Effects of the Invention]

It reaches the point where it can do genetic therapeutic of muscular dystrophy where the immune reaction is less due to gene of this invention and using gene introduction medium for genetic therapeutic of muscular dystrophy.

[0080]

Sequence Number: 1

Length of sequence: 3748

Form of sequence: nucleic acid

Number of strands: Both morphological form (both)

Topology: straight chain

Kind of sequence: Feature: active-site of cDNA to mRNA arrangement

Arrangement

CGGCCGCTCT AGAGGATCCC CGGGTACCGA GCTCGAATTC CGGAACTCCC GGAGAAAAAC 60

GAATAGGAAA AACTGAAGTG TTACTTTTTT TAAAGCTGCT GAAGTTTGTT GGTTTCTCAT 120

TGTTTTTAAG CCTACTGGAG CAATAAAGTT TGAAGAACTT TTACCAGGTT TTTTTTATCG 180

CTGCCTTGAT ATACACTTTT CAAAATGCTT TGGTGGGAAG AAGTAGAGGA CTGTTATGAA 240

AGAGAAGATG TTCAAAAGAA AACATTCACA AAATGGGTAA ATGCACAATT TTCTAAGTTT 300

GGGAAGCAGC ATATTGAGAA CCTCTTCAGT GACCTACAGG ATGGGAGGCG CCTCCTAGAC 360

CTCCTCGAAG GCCTGACAGG GCAAAAACTG CCAAAAGAAA AAGGATCCAC AAGAGTTCAT 420

GCCCTGAACA ATGTCAACAA GGCACTGCGG GTTTTGCAGA ACAATAATGT TGATTTAGTG 480

AATATTGGAA GTACTGACAT CGTAGATGGA AATCATAAAC TGACTCTTGG TTTGATTTGG 540

AATATAATCC TCCACTGGCA GGTCAAAAAT GTAATGAAAA ATATCATGGC TGGATTGCAA 600

CAAACCAACA GTGAAAAGAT TCTCCTGAGC TGGGTCCGAC AATCAACTCG TAATTATCCA 660

CAGGTTAATG TAATCAACTT CACCACCAGC TGGTCTGATG GCCTGGCTTT GAATGCTCTC 720

ATCCATAGTC ATAGGCCAGA CCTATTTGAC TGGAATAGTG TGGTTTGCCA GCAGTCAGCC 780

ACACAACGAC TGGAACATGC ATTCAACATC GCCAGATATC AATTAGGCAT AGAGAAACTA 840

CTCGATCCTG AAGATGTTGA TACCACCTAT CCAGATAAGA AGTCCATCTT AATGTACATC 900

ACATCACTCT TCCAAGTTTT GCCTCAACAA GTGAGCATTG AAGCCATCCA GGAAGTGGAA 960

ATGTTGCCAA GGCCACCTAA AGTGACTAAA GAAGAACATT TTCAGTTACA TCATCAAATG 1020

CACTATTCTC AACAGATCAC GGTCAGTCTA GCACAGGGAT ATGAGAGAAC TTCTTCCCCT 1080

AAGCCTCGAT TCAAGAGCTA TGCCTACACA CAGGCTGCTT ATGTCACCAC CTCTGACCCT 1140

ACACGGAGCC CATTTCCTTC ACAGCATTTG GAAGCTCCTG AAGACAAGTC ATTTGGCAGT 1200

TCATTGATGG AGAGTGAAGT AAACCTGGAC CGTTATCAAA CAGCTTTAGA AGAAGTATTA 1260

TCGTGGCTTC TTTCTGCTGA GGACACATTG CAAGCACAAG GAGAGATTTC TAATGATGTG 1320

GAAGTGGTGA AAGACCAGTT TCATACTCAT GAGGGGTACA TGATGGATTT GACAGCCCAT 1380

CAGGGCCGGG TTGGTAATAT TCTACAATTG GGAAGTAAGC TGATTGGAAC AGGAAAATTA 1440

TCAGAAGATG AAGAAACTGA AGTACAAGAG CAGATGAATC TCCTAAATTC AAGATGGAAG 1500

CTTCTGCAGG TGGCCGTCGA GGACCGAGTC AGGCAGCTGC ATGAAGCCCA CAGGGACTTT 1560

GGTCCAGCAT CTCAGCACTT TCTTTCCACG TCTGTCCAGG GTCCCTGGGA GAGAGCCATC 1620

TCGCCAAACA AAGTGCCCTA CTATATCAAC CACGAGACTC AAACAACTTG CTGGGACCAT 1680

CCCAAAATGA CAGAGCTCTA CCAGTCTTTA GCTGACCTGA ATAATGTCAG ATTCTCAGCT 1740

TATAGGACTG CCATGAAACT CCGAAGACTG CAGAAGGCCC TTTGCTTGGA TCTCTTGAGC 1800

CTGTCAGCTG CATGTGATGC CTTGGACCAG CACAACCTCA AGCAAAATGA CCAGCCCATG 1860

GATATCCTGC AGATTATTAA TTGTTTGACC ACTATTTATG ACCGCCTGGA GCAAGAGCAC 1920

AACAATTTGG TCAACGTCCC TCTCTGCGTG GATATGTGTC TGAACTGGCT GCTGAATGTT 1980

TATGATACGG GACGAACAGG GAGGATCCGT GTCCTGTCTT TTAAAACTGG CATCATTTCC 2040

CTGTGTAAAG CACATTTGGA AGACAAGTAC AGATACCTTT TCAAGCAAGT GGCAAGTTCA 2100

ACAGGATTTT GTGACCAGCG CAGGCTGGGC CTCCTTCTGC ATGATTCTAT CCAAATTCCA 2160

AGACAGTTGG GTGAAGTTGC ATCCTTTGGG GGCAGTAACA TTGAGCCAAG TGTCCGGAGC 2220

TGCTTCCAAT TTGCTAATAA TAAGCCAGAG ATCGAAGCGG CCCTCTTCCT AGACTGGATG 2280

AGACTGGAAC CCCAGTCCAT GGTGTGGCTG CCCGTCCTGC ACAGAGTGGC TGCTGCAGAA 2340

ACTGCCAAGC ATCAGGCCAA ATGTAACATC TGCAAAGAGT GTCCAATCAT TGGATTCAGG 2400

TACAGGAGTC TAAAGCACTT TAATTATGAC ATCTGCCAAA GCTGCTTTTT TTCTGGTCGA 2460

GTTGCAAAAG GCCATAAAAT GCACTATCCC ATGGTGGAAT ATTGCACTCC GACTACATCA 2520

GGAGAAGATG TTCGAGACTT TGCCAAGGTA CTAAAAAACA AATTTCGAAC CAAAAGGTAT 2580

TTTGCGAAGC ATCCCCGAAT GGGCTACCTG CCAGTGCAGA CTGTCTTAGA GGGGGACAAC 2640

ATGGAAACTC CCGTTACTCT GATCAACTTC TGGCCAGTAG ATTCTGCGCC TGCCTCGTCC 2700

CCTCAGCTTT CACACGATGA TACTCATTCA CGCATTGAAC ATTATGCTAG CAGGCTAGCA 2760

GAAATGGAAA ACAGCAATGG ATCTTATCTA AATGATAGCA TCTCTCCTAA TGAGAGCATA 2820

GATGATGAAC ATTTGTTAAT CCAGCATTAC TGCCAAAGTT TGAACCAGGA CTCCCCCCTG 2880

AGCCAGCCTC GTAGTCCTGC CCAGATCTTG ATTTCCTTAG AGAGTGAGGA AAGAGGGGAG 2940

CTAGAGAGAA TCCTAGCAGA TCTTGAGGAA GAAAACAGGA ATCTGCAAGC AGAATATGAC 3000

CGTCTAAAGC AGCAGCACGA ACATAAAGGC CTGTCCCCAC TGCCGTCCCC TCCTGAAATG 3060

ATGCCCACCT CTCCCCAGAG TCCCCGGGAT GCTGAGCTCA TTGCTGAGGC CAAGCTACTG 3120

CGTCAACAC AAAGGCCGCC TGGAAGCCAG GATGCAAATC CTGGAAGACC ACAATAAACAG 3180

CTGGAGTCA CAGTTACACA GGCTAAGGCA GCTGCTGGAG CAACCCCAGG CAGAGGCCAAA 3240

GTGAATGGC ACAACGGTGT CCTCTCCTTC TACCTCTCTA CAGAGGTCCG ACAGCAGTCAG 3300

CCTATGCTG CTCCGAGTGG TTGGCAGTCA AACTTCGGAC TCCATGGGTG AGGAAGATCTT 3360

CTCAGTCCT CCCCAGGACA CAAGCACAGG GTTAGAGGAG GTGATGGAGC AACTCAACAAC 3420

TCCTTCCCT AGTTCAAGAG GAAGAAATAC CCCTGGAAAG CCAATGAGAG AGGACACAATG 3480

TAGGAAGTC TTTTCCACAT GGCAGATGAT TTGGGCAGAG CGATGGAGTC CTTAGTATCAG 3540

TCATGACAG ATGAAGAAGG AGCAGAATAA ATGTTTTACA ACTCCTGATT CCCGCATGGTT 3600

TTTATAATA TTCATACAAC AAAGAGGATT AGACAGTAAG AGTTTACAAG AAATAAATCTA 3660

TATTTTTGT GAAGGGTAGT GGTATTATAC TGTAGATTTC AGTAGTTTCT AAGTCTGTTAT 3720

GTTTTGTTG GGGATCCTCT AGAGTCGA 3748

Sequence Number: 2

Length of sequence: 1092

Form of sequence: amino acid

Topology: straight chain

Kind of sequence: protein

Arrangement

Met Leu Trp Trp Glu Glu Val Glu Asp Cys Tyr Glu Arg Glu Asp 15

Val Gln Lys Lys Thr Phe Thr Lys Trp Val Asn Ala Gln Phe Ser 30

Lys Phe Gly Lys Gln His Ile Glu Asn Leu Phe Ser Asp Leu Gln 45

Asp Gly Arg Arg Leu Leu Asp Leu Leu Glu Gly Leu Thr Gly Gln 60

Lys Leu Pro Lys Glu Lys Gly Ser Thr Arg Val His Ala Leu Asn 75

Asn Val Asn Lys Ala Leu Arg Val Leu Gln Asn Asn Asn Val Asp 90

Leu Val Asn Ile Gly Ser Thr Asp Ile Val Asp GlyA sn His Lys 105

Leu Thr Leu Gly Leu Ile Trp Asn Ile Ile Leu His Trp Gln Val 120

Lys Asn Val Met Lys Asn Ile Met Ala Gly Leu Gln Gln Thr Asn 135

Ser Glu Lys Ile Leu Leu Ser Trp Val Arg Gln Ser Thr Arg Asn 150

Tyr Pro Gln Val Asn Val Ile Asn Phe Thr Thr Ser Trp Ser Asp 165

Gly Leu Ala Leu Asn Ala Leu Ile His Ser His Arg Pro Asp Leu 180

Phe Asp Trp Asn Ser Val Val Cys Gln Gln Ser Ala Thr Gln Arg 195

Leu Glu His Ala Phe Asn Ile Ala Arg Tyr Gln Leu Gly Ile Glu 210

Lys Leu Leu Asp Pro Glu Asp Val Asp Thr Thr Tyr Pro Asp Lys 225

Lys Ser Ile Leu Met Tyr Ile Thr Ser Leu Phe Gln Val Leu Pro 240

Gln Gln Val Ser Ile Glu Ala Ile Gln Glu Val Glu Met Leu Pro 255

Arg Pro Pro Lys Val Thr Lys Glu Glu His Phe Gln Leu His His 270

Gln Met His Tyr Ser Gln Gln Ile Thr Val Ser Leu Ala Gln Gly 285

Tyr Glu Arg Thr Ser Ser Pro Lys Pro Arg Phe Lys Ser Tyr Ala 300

Tyr Thr Gln Ala Ala Tyr Val Thr Thr Ser Asp Pro Thr Arg Ser 315

Pro Phe Pro Ser Gln His Leu Glu Ala Pro Glu Asp Lys Ser Phe 330

Gly Ser Ser Leu Met Glu Ser Glu Val Asn Leu Asp Arg Tyr Gln 345

Thr Ala Leu Glu Glu Val Leu Ser Trp Leu Leu Ser Ala Glu Asp 360

Thr Leu Gln Ala Gln Gly Glu Ile Ser Asn Asp Val Glu Val Val 375

Lys Asp Gln Phe His Thr His Glu Gly Tyr Met Met Asp Leu Thr 390

Ala His Gln Gly Arg Val Gly Asn Ile Leu Gln Leu Gly Ser Lys 405

Leu Ile Gly Thr Gly Lys Leu Ser Glu Asp Glu Glu Thr Glu Val 420

Gln Glu Gln Met Asn Leu Leu Asn Ser Arg Trp Lys Leu Leu Gln 435

Val Ala Val Glu Asp Arg Val Arg Gln Leu His Glu Ala His Arg 450

Asp Phe Gly Pro Ala Ser Gln His Phe Leu Ser Thr Ser Val Gln 465

Gly Pro Trp Glu Arg Ala Ile Ser Pro Asn Lys Val Pro Tyr Tyr 480

Ile Asn His Glu Thr Gln Thr Thr Cys Trp Asp His Pro Lys Met 495

Thr Glu Leu Tyr Gln Ser Leu Ala Asp Leu Asn Asn Val Arg Phe 510

Ser Ala Tyr Arg Thr Ala Met Lys Leu Arg Arg Leu Gln Lys Ala 525

Leu Cys Leu Asp Leu Leu Ser Leu Ser Ala Ala Cys Asp Ala Leu 540

Asp Gln His Asn Leu Lys Gln Asn Asp Gln Pro Met Asp Ile Leu 555

Gln Ile Ile Asn Cys Leu Thr Thr Ile Tyr Asp Arg Leu Glu Gln 570

Glu His Asn Asn Leu Val Asn Val Pro Leu Cys Val Asp Met Cys 585

Leu Asn Trp Leu Leu Asn Val Tyr Asp Thr Gly Arg Thr Gly Arg 600

Ile Arg Val Leu Ser Phe Lys Thr Gly Ile Ile Ser Leu Cys Lys 615

Ala His Leu Glu Asp Lys Tyr Arg Tyr Leu Phe Lys Gln Val Ala 630

Ser Ser Thr Gly Phe Cys Asp Gln Arg Arg Leu Gly Leu Leu Leu 645

His Asp Ser Ile Gln Ile Pro Arg Gln Leu Gly Glu Val Ala Ser 660

Phe Gly Gly Ser Asn Ile Glu Pro Ser Val Arg Ser Cys Phe Gln 675

Phe Ala Asn Asn Lys Pro Glu Ile Glu Ala Ala Leu Phe Leu Asp 690

Trp Met Arg Leu Glu Pro Gln Ser Met Val Trp Leu Pro Val Leu 705

His Arg Val Ala Ala Ala Glu Thr Ala Lys His Gln Ala Lys Cys 720

Asn Ile Cys Lys Glu Cys Pro Ile Ile Gly Phe Arg Tyr Arg Ser 735

Leu Lys His Phe Asn Tyr Asp Ile Cys Gln Ser Cys Phe Phe Ser 750

Gly Arg Val Ala Lys Gly His Lys Met His Tyr Pro Met Val Glu 765

Tyr Cys Thr Pro Thr Thr Ser Gly Glu Asp Val Arg Asp Phe Ala 780

Lys Val Leu Lys Asn Lys Phe Arg Thr Lys Arg Tyr Phe Ala Lys 795

His Pro Arg Met Gly Tyr Leu Pro Val Gln Thr Val Leu Glu Gly 810

Asp Asn Met Glu Thr Pro Val Thr Leu Ile Asn Phe Trp Pro Val 825

Asp Ser Ala Pro Ala Ser Ser Pro Gln Leu Ser His Asp Asp Thr 840

His Ser Arg Ile Glu His Tyr Ala Ser Arg Leu Ala Glu Met Glu 855

Asn Ser Asn Gly Ser Tyr Leu Asn Asp Ser Ile Ser Pro Asn Glu 870

Ser Ile Asp Asp Glu His Leu Leu Ile Gln His Tyr Cys Gln Ser 885

Leu Asn Gln Asp Ser Pro Leu Ser Gln Pro Arg Ser Pro Ala Gln 900

Ile Leu Ile Ser Leu Glu Ser Glu Glu Arg Gly Glu Leu Glu Arg 915

Ile Leu Ala Asp Leu Glu Glu Glu Asn Arg Asn Leu Gln Ala Glu 930

Tyr Asp Arg Leu Lys Gln Gln His Glu His Lys Gly Leu Ser Pro 945

Leu Pro Ser Pro Pro Glu Met Met Pro Thr Ser Pro Gln Ser Pro 960

Arg Asp Ala Glu Leu Ile Ala Glu Ala Lys Leu Leu Arg Gln His 975

Lys Gly Arg Leu Glu Ala Arg Met Gln Ile Leu Glu Asp His Asn 990

Lys Gln Leu Glu Ser Gln Leu His Arg Leu Arg Gln Leu Leu Glu 1005

Gln Pro Gln Ala Glu Ala Lys Val Asn Gly Thr Thr Val Ser Ser 1020

Pro Ser Thr Ser Leu Gln Arg Ser Asp Ser Ser Gln Pro Met Leu 1035

Leu Arg Val Val Gly Ser Gln Thr Ser Asp Ser Met Gly Glu Glu 1050

Asp Leu Leu Ser Pro Pro Gln Asp Thr Ser Thr Gly Leu Glu Glu 1065

Val Met Glu Gln Leu Asn Asn Ser Phe Pro Ser Ser Arg Gly Arg 1080

Asn Thr Pro Gly Lys Pro Met Arg Glu Asp Thr Met 1092

Sequence Number: 3

Length of sequence: 4402

Form of sequence: nucleic acid

Number of strands: Both morphological form (both)

Topology: straight chain

Kind of sequence: Feature: active-site of cDNA to mRNA arrangement

Arrangement

CGGCCGCTCT AGAGGATCCC CGGGTACCGA GCTCGAATTC CGGAACTCCC GGAGAAAAAC 60

GAATAGGAAA AACTGAAGTG TTACTTTTTT TAAAGCTGCT GAAGTTTGTT GGTTTCTCAT 120

TGTTTTTAAG CCTACTGGAG CAATAAAGTT TGAAGAACTT TTACCAGGTT TTTTTTATCG 180

CTGCCTTGAT ATACACTTTT CAAAATGCTT TGGTGGGAAG AAGTAGAGGA CTGTTATGAA 240

AGAGAAGATG TTCAAAAGAA AACATTCACA AAATGGGTAA ATGCACAATT TTCTAAGTTT 300

GGGAAGCAGC ATATTGAGAA CCTCTTCAGT GACCTACAGG ATGGGAGGCG CCTCCTAGAC 360

CTCCTCGAAG GCCTGACAGG GCAAAAACTG CCAAAAGAAA AAGGATCCAC AAGAGTTCAT 420

GCCCTGAACA ATGTCAACAA GGCACTGCGG GTTTTGCAGA ACAATAATGT TGATTTAGTG 480

AATATTGGAA GTACTGACAT CGTAGATGGA AATCATAAAC TGACTCTTGG TTTGATTTGG 540

AATATAATCC TCCACTGGCA GGTCAAAAAT GTAATGAAAA ATATCATGGC TGGATTGCAA 600

CAAACCAACA GTGAAAAGAT TCTCCTGAGC TGGGTCCGAC AATCAACTCG TAATTATCCA 660

CAGGTTAATG TAATCAACTT CACCACCAGC TGGTCTGATG GCCTGGCTTT GAATGCTCTC 720

ATCCATAGTC ATAGGCCAGA CCTATTTGAC TGGAATAGTG TGGTTTGCCA GCAGTCAGCC 780

ACACAACGAC TGGAACATGC ATTCAACATC GCCAGATATC AATTAGGCAT AGAGAAACTA 840

CTCGATCCTG AAGATGTTGA TACCACCTAT CCAGATAAGA AGTCCATCTT AATGTACATC 900

ACATCACTCT TCCAAGTTTT GCCTCAACAA GTGAGCATTG AAGCCATCCA GGAAGTGGAA 960

ATGTTGCCAA GGCCACCTAA AGTGACTAAA GAAGAACATT TTCAGTTACA TCATCAAATG 1020

CACTATTCTC AACAGATCAC GGTCAGTCTA GCACAGGGAT ATGAGAGAAC TTCTTCCCCT 1080

AAGCCTCGAT TCAAGAGCTA TGCCTACACA CAGGCTGCTT ATGTCACCAC CTCTGACCCT 1140

ACACGGAGCC CATTTCCTTC ACAGCATTTG GAAGCTCCTG AAGACAAGTC ATTTGGCAGT 1200

TCATTGATGG AGAGTGAAGT AAACCTGGAC CGTTATCAAA CAGCTTTAGA AGAAGTATTA 1260

TCGTGGCTTC TTTCTGCTGA GGACACATTG CAAGCACAAG GAGAGATTTC TAATGATGTG 1320

GAAGTGGTGA AAGACCAGTT TCATACTCAT GAGGGGTACA TGATGGATTT GACAGCCCAT 1380

CAGGGCCGGG TTGGTAATAT TCTACAATTG GGAAGTAAGC TGATTGGAAC AGGAAAATTA 1440

TCAGAAGATG AAGAAACTGA AGTACAAGAG CAGATGAATC TCCTAAATTC AAGATGGGAA 1500

TGCCTCAGGG TAGCTAGCAT GGAAAAACAA AGCAATTTAC ATAGAGTTTT AATGGATCTC 1560

CAGAATCAGA AACTGAAAGA GTTGAATGAC TGGCTAACAA AAACAGAAGA AAGAACAAGG 1620

AAAATGGAGG AAGAGCCTCT TGGACCTGAT CTTGAAGACC TAAAACGCCA AGTACAACAA 1680

CATAAGGTGC TTCAAGAAGA TCTAGAACAA GAACAAGTCA GGGTCAATTCT CTCACTCAC 1740

ATGGTGGTGG TAGTTGATGA ATCTAGTGGA GATCACGCAA CTGCTGCTTTG GAAGAACAA 1800

CTTAAGGAGG TCAATACTGA GTGGGAAAAA TTGAACCTGC ACTCCGCTGAC TGGCAGAGA 1860

AAAATAGATG AGACCCTTGA AAGACTCCAG GAACTTCAAG AGGCCACGGAT GAGCTGGAC 1920

CTCAAGCTGC GCCAAGCTGA GGTGATCAAG GGATCCTGGC AGCCCGTGGGC GATCTCCTC 1980

ATTGACTCTC TCCAAGATCA CCTCGAGAAA GTCAAGGCAC TTCGAGGAGAA ATTGCGCCT 2040

CTGAAAGAGA ACGTGAGCCA CGTCAATGAC CTTGCTCGCC AGCTTACCACT TTGGGCATT 2100

CAGCTCTCAC CGTATAACCT CAGCACTCTG GAAGACCTGA ACACCAGATGG AAGCTTCTG 2160

CAGGTGGCCG TCGAGGACCG AGTCAGGCAG CTGCATGAAG CCCACAGGGAC TTTGGTCCA 2220

GCATCTCAGC ACTTTCTTTC CACGTCTGTC CAGGGTCCCT GGGAGAGAGCC ATCTCGCCA 2280

AACAAAGTGC CCTACTATAT CAACCACGAG ACTCAAACAA CTTGCTGGGAC CATCCCAAA 2340

ATGACAGAGC TCTACCAGTC TTTAGCTGAC CTGAATAATG TCAGATTCTCA GCTTATAGG 2400

ACTGCCATGA AACTCCGAAG ACTGCAGAAG GCCCTTTGCT TGGATCTCTTG AGCCTGTCA 2460

GCTGCATGTG ATGCCTTGGA CCAGCACAAC CTCAAGCAAA ATGACCAGCCC ATGGATATC 2520

CTGCAGATTA TTAATTGTTT GACCACTATT TATGACCGCC TGGAGCAAGAG CACAACAAT 2580

TTGGTCAACG TCCCTCTCTG CGTGGATATG TGTCTGAACT GGCTGCTGAAT GTTTATGAT 2640

ACGGGACGAA CAGGGAGGAT CCGTGTCCTG TCTTTTAAAA CTGGCATCATT TCCCTGTGT 2700

AAAGCACATT TGGAAGACAA GTACAGATAC CTTTTCAAGC AAGTGGCAAGT TCAACAGGA 2760

TTTTGTGACC AGCGCAGGCT GGGCCTCCTT CTGCATGATT CTATCCAAATT CCAAGACAG 2820

TTGGGTGAAG TTGCATCCTT TGGGGGCAGT AACATTGAGC CAAGTGTCCGG AGCTGCTTC 2880

CAATTTGCTA ATAATAAGCC AGAGATCGAA GCGGCCCTCT TCCTAGACTGG ATGAGACTG 2940

GAACCCCAGT CCATGGTGTG GCTGCCCGTC CTGCACAGAG TGGCTGCTGCA GAAACTGCC 3000

AAGCATCAGG CCAAATGTAA CATCTGCAAA GAGTGTCCAA TCATTGGATTC AGGTACAGG 3060

AGTCTAAAGC ACTTTAATTA TGACATCTGC CAAAGCTGCT TTTTTTCTGGT CGAGTTGCA 3120

AAAGGCCATA AAATGCACTA TCCCATGGTG GAATATTGCA CTCCGACTACA TCAGGAGAA 3180

GATGTTCGAG ACTTTGCCAA GGTACTAAAA AACAAATTTC GAACCAAAAGG TATTTTGCG 3240

AAGCATCCCC GAATGGGCTA CCTGCCAGTG CAGACTGTCT TAGAGGGGGAC AACATGGAA 3300

ACTCCCGTTA CTCTGATCAA CTTCTGGCCA GTAGATTCTG CGCCTGCCTCG TCCCCTCAG 3360

CTTTCACACG ATGATACTCA TTCACGCATT GAACATTATG CTAGCAGGCTA GCAGAAATG 3420

GAAAACAGCA ATGGATCTTA TCTAAATGAT AGCATCTCTC CTAATGAGAGC ATAGATGAT 3480

GAACATTTGT TAATCCAGCA TTACTGCCAA AGTTTGAACC AGGACTCCCCC CTGAGCCAG 3540

CCTCGTAGTC CTGCCCAGAT CTTGATTTCC TTAGAGAGTG AGGAAAGAGGG GAGCTAGAG 3600

AGAATCCTAG CAGATCTTGA GGAAGAAAAC AGGAATCTGC AAGCAGAATAT GACCGTCTA 3660

AAGCAGCAGC ACGAACATAA AGGCCTGTCC CCACTGCCGT CCCCTCCTGAA ATGATGCCC 3720

ACCTCTCCCC AGAGTCCCCG GGATGCTGAG CTCATTGCTG AGGCCAAGCTA CTGCGTCAA 3780

CACAAAGGCC GCCTGGAAGC CAGGATGCAA ATCCTGGAAG ACCACAATAAA CAGCTGGAG 3840

TCACAGTTAC ACAGGCTAAG GCAGCTGCTG GAGCAACCCC AGGCAGAGGCC AAAGTGAAT 3900

GGCACAACGG TGTCCTCTCC TTCTACCTCT CTACAGAGGT CCGACAGCAGT CAGCCTATG 3960

CTGCTCCGAG TGGTTGGCAG TCAAACTTCG GACTCCATGG GTGAGGAAGAT CTTCTCAGT 4020

CCTCCCCAGG ACACAAGCAC AGGGTTAGAG GAGGTGATGG AGCAACTCAAC AACTCCTTC 4080

CCTAGTTCAA GAGGAAGAAA TACCCCTGGA AAGCCAATGA GAGAGGACACA ATGTAGGAA 4140

GTCTTTTCCA CATGGCAGAT GATTTGGGCA GAGCGATGGA GTCCTTAGTAT CAGTCATGA 4200

CAGATGAAGA AGGAGCAGAA TAAATGTTTT ACAACTCCTG ATTCCCGCATG GTTTTTATA 4260

ATATTCATAC AACAAAGAGG ATTAGACAGT AAGAGTTTAC AAGAAATAAAT CTATATTTT 4320

TGTGAAGGGT AGTGGTATTA TACTGTAGAT TTCAGTAGTT TCTAAGTCTGT TATTGTTTT 4380

GTTGGGGATC CTCTAGAGTC GA 4402

Sequence Number: 4

Length of sequence: 1,310

Form of sequence: amino acid

Topology: straight chain
Kind of sequence: protein
Arrangement

Met Leu Trp Trp Glu Glu Val Glu Asp Cys Tyr Glu Arg Glu Asp 15

Val Gln Lys Lys Thr Phe Thr Lys Trp Val Asn Ala Gln Phe Ser 30

Lys Phe Gly Lys Gln His Ile Glu Asn Leu Phe Ser Asp Leu Gln 45

Asp Gly Arg Arg Leu Leu Asp Leu Leu Glu Gly Leu Thr Gly Gln 60

Lys Leu Pro Lys Glu Lys Gly Ser Thr Arg Val His Ala Leu Asn 75

Asn Val Asn Lys Ala Leu Arg Val Leu Gln Asn Asn Asn Val Asp 90

Leu Val Asn Ile Gly Ser Thr Asp Ile Val Asp Gly Asn His Lys 105

Leu Thr Leu Gly Leu Ile Trp Asn Ile Ile Leu His Trp Gln Val 120

Lys Asn Val Met Lys Asn Ile Met Ala Gly Leu Gln Gln Thr Asn 135

Ser Glu Lys Ile Leu Leu Ser Trp Val Arg Gln Ser Thr Arg Asn 150

Tyr Pro Gln Val Asn Val Ile Asn Phe Thr Thr Ser Trp Ser Asp 165

Gly Leu Ala Leu Asn Ala Leu Ile His Ser His Arg Pro Asp Leu 180

Phe Asp Trp Asn Ser Val Val Cys Gln Gln Ser Ala Thr Gln Arg 195

Leu Glu His Ala Phe Asn Ile Ala Arg Tyr Gln Leu Gly Ile Glu 210

Lys Leu Leu Asp Pro Glu Asp Val Asp Thr Thr Tyr Pro Asp Lys 225

Lys Ser Ile Leu Met Tyr Ile Thr Ser Leu Phe Gln Val Leu Pro 240

Gln Gln Val Ser Ile Glu Ala Ile Gln Glu Val Glu Met Leu Pro 255

Arg Pro Pro Lys Val Thr Lys Glu Glu His Phe Gln Leu His His 270

Gln Met His Tyr Ser Gln Gln Ile Thr Val Ser Leu Ala Gln Gly 285

Tyr Glu Arg Thr Ser Ser Pro Lys Pro Arg Phe Lys Ser Tyr Ala 300

Tyr Thr Gln Ala Ala Tyr Val Thr Thr Ser Asp Pro Thr Arg Ser 315

Pro Phe Pro Ser Gln His Leu Glu Ala Pro Glu Asp Lys Ser Phe 330

Gly Ser Ser Leu Met Glu Ser Glu Val Asn Leu Asp Arg Tyr Gln 345

Thr Ala Leu Glu Glu Val Leu Ser Trp Leu Leu Ser Ala Glu Asp 360

Thr Leu Gln Ala Gln Gly Glu Ile Ser Asn Asp Val Glu Val Val 375

Lys Asp Gln Phe His Thr His Glu Gly Tyr Met Met Asp Leu Thr 390

Ala His Gln Gly Arg Val Gly Asn Ile Leu Gln Leu Gly Ser Lys 405

Leu Ile Gly Thr Gly Lys Leu Ser Glu Asp Glu Glu Thr Glu Val 420

Gln Glu Gln Met Asn Leu Leu Asn Ser Arg Trp Glu Cys Leu Arg 435

Val Ala Ser Met Glu Lys Gln Ser Asn Leu His Arg Val Leu Met 450

Asp Leu Gln Asn Gln Lys Leu Lys Glu Leu Asn Asp Trp Leu Thr 465

Lys Thr Glu Glu Arg Thr Arg Lys Met Glu Glu Glu Pro Leu Gly 480

Pro Asp Leu Glu Asp Leu Lys Arg Gln Val Gln Gln His Lys Val 495

Leu Gln Glu Asp Leu Glu Gln Glu Gln Val Arg Val Asn Ser Leu 510

Thr His Met Val Val Val Val Asp Glu Ser Ser Gly Asp His Ala 525

Thr Ala Ala Leu Glu Glu Gln Leu Lys Glu Val Asn Thr Glu Trp 540

Glu Lys Leu Asn Leu His Ser Ala Asp Trp Gln Arg Lys Ile Asp 555

Glu Thr Leu Glu Arg Leu Gln Glu Leu Gln Glu Ala Thr Asp Glu 570

Leu Asp Leu Lys Leu Arg Gln Ala Glu Val Ile Lys Gly Ser Trp 585

Gln Pro Val Gly Asp Leu Leu Ile Asp Ser Leu Gln Asp His Leu 600

Glu Lys Val Lys Ala Leu Arg Gly Glu Ile Ala Pro Leu Lys Glu 615

Asn Val Ser His Val Asn Asp Leu Ala Arg Gln Leu Thr Thr Leu 630

Gly Ile Gln Leu Ser Pro Tyr Asn Leu Ser Thr Leu Glu Asp Leu 645

Asn Thr Arg Trp Lys Leu Leu Gln Val Ala Val Glu Asp Arg Val 660

Arg Gln Leu His Glu Ala His Arg Asp Phe Gly Pro Ala Ser Gln 675

His Phe Leu Ser Thr Ser Val Gln Gly Pro Trp Glu Arg Ala Ile 690

Ser Pro Asn Lys Val Pro Tyr Tyr Ile Asn His Glu Thr Gln Thr 705

Thr Cys Trp Asp His Pro Lys Met Thr Glu Leu Tyr Gln Ser Leu 720

Ala Asp Leu Asn Asn Val Arg Phe Ser Ala Tyr Arg Thr Ala Met 735

Lys Leu Arg Arg Leu Gln Lys Ala Leu Cys Leu Asp Leu Leu Ser 750

Leu Ser Ala Ala Cys Asp Ala Leu Asp Gln His Asn Leu Lys Gln 765

Asn Asp Gln Pro Met Asp Ile Leu Gln Ile Ile Asn Cys Leu Thr 780

Thr Ile Tyr Asp Arg Leu Glu Gln Glu His Asn Asn Leu Val Asn 795

Val Pro Leu Cys Val Asp Met Cys Leu Asn Trp Leu Leu Asn Val 810

Tyr Asp Thr Gly Arg Thr Gly Arg Ile Arg Val Leu Ser Phe Lys 825

Thr Gly Ile Ile Ser Leu Cys Lys Ala His Leu Glu Asp Lys Tyr 840

Arg Tyr Leu Phe Lys Gln Val Ala Ser Ser Thr Gly Phe Cys Asp 855

Gln Arg Arg Leu Gly Leu Leu Leu His Asp Ser Ile Gln Ile Pro 870

Arg Gln Leu Gly Glu Val Ala Ser Phe Gly Gly Ser Asn Ile Glu 885

Pro Ser Val Arg Ser Cys Phe Gln Phe Ala Asn Asn Lys Pro Glu 900

Ile Glu Ala Ala Leu Phe Leu Asp Trp Met Arg Leu Glu Pro Gln 915

Ser Met Val Trp Leu Pro Val Leu His Arg Val Ala Ala Ala Glu 930

Thr Ala Lys His Gln Ala Lys Cys Asn Ile Cys Lys Glu Cys Pro 945

Ile Ile Gly Phe Arg Tyr Arg Ser Leu Lys His Phe Asn Tyr Asp 960

Ile Cys Gln Ser Cys Phe Phe Ser Gly Arg Val Ala Lys Gly His 975

Lys Met His Tyr Pro Met Val Glu Tyr Cys Thr Pro Thr Thr Ser 990

Gly Glu Asp Val Arg Asp Phe Ala Lys Val Leu Lys Asn Lys Phe 1005

Arg Thr Lys Arg Tyr Phe Ala Lys His Pro Arg Met Gly Tyr Leu 1020

Pro Val Gln Thr Val Leu Glu Gly Asp Asn Met Glu Thr Pro Val 1035

Thr Leu Ile Asn Phe Trp Pro Val Asp Ser Ala Pro Ala Ser Ser 1050

Pro Gln Leu Ser His Asp Asp Thr His Ser Arg Ile Glu His Tyr 1065

Ala Ser Arg Leu Ala Glu Met Glu Asn Ser Asn Gly Ser Tyr Leu 1080

Asn Asp Ser Ile Ser Pro Asn Glu Ser Ile Asp Asp Glu His Leu 1095

Leu Ile Gln His Tyr Cys Gln Ser Leu Asn Gln Asp Ser Pro Leu 1110

Ser Gln Pro Arg Ser Pro Ala Gln Ile Leu Ile Ser Leu Glu Ser 1125

Glu Glu Arg Gly Glu Leu Glu Arg Ile Leu Ala Asp Leu Glu Glu 1140

Glu Asn Arg Asn Leu Gln Ala Glu Tyr Asp Arg Leu Lys Gln Gln 1155

His Glu His Lys Gly Leu Ser Pro Leu Pro Ser Pro Pro Glu Met 1170

Met Pro Thr Ser Pro Gln Ser Pro Arg Asp Ala Glu Leu Ile Ala 1185

Glu Ala Lys Leu Leu Arg Gln His Lys Gly Arg Leu Glu Ala Arg 1200

Met Gln Ile Leu Glu Asp His Asn Lys Gln Leu Glu Ser Gln Leu 1215

His Arg Leu Arg Gln Leu Leu Glu Gln Pro Gln Ala Glu Ala Lys 1230

Val Asn Gly Thr Thr Val Ser Ser Pro Ser Thr Ser Leu Gln Arg 1245

Ser Asp Ser Ser Gln Pro Met Leu Leu Arg Val Val Gly Ser Gln 1260

Thr Ser Asp Ser Met Gly Glu Glu Asp Leu Leu Ser Pro Pro Gln 1275

Asp Thr Ser Thr Gly Leu Glu Glu Val Met Glu Gln Leu Asn Asn 1290

Ser Phe Pro Ser Ser Arg Gly Arg Asn Thr Pro Gly Lys Pro Met 1305

Arg Glu Asp Thr Met 1310

Sequence Number: 5

Length of sequence: 4402

Form of sequence: nucleic acid

Number of strands: Both morphological form (both)

Topology: straight chain

Kind of sequence: Feature: active-site of cDNA to mRNA arrangement

Arrangement

CGGCCGCTCT AGAGGATCCC CGGGTACCGA GCTCGAATTC CGGAACTCCC GGAGAAAAAC 60

GAATAGGAAA AACTGAAGTG TTACTTTTTT TAAAGCTGCT GAAGTTTGTT GGTTTCTCAT 120

TGTTTTTAAG CCTACTGGAG CAATAAAGTT TGAAGAACTT TTACCAGGTT TTTTTTATCG 180

CTGCCTTGAT ATACACTTTT CAAAATGCTT TGGTGGGAAG AAGTAGAGGA CTGTTATGAA 240

AGAGAAGATG TTCAAAAGAA AACATTCACA AAATGGGTAA ATGCACAATT TTCTAAGTTT 300

GGGAAGCAGC ATATTGAGAA CCTCTTCAGT GACCTACAGG ATGGGAGGCG CCTCCTAGAC 360

CTCCTCGAAG GCCTGACAGG GCAAAAACTG CCAAAAGAAA AAGGATCCAC AAGAGTTCAT 420

GCCCTGAACA ATGTCAACAA GGCACTGCGG GTTTTGCAGA ACAATAATGT TGATTTAGTG 480

AATATTGGAA GTACTGACAT CGTAGATGGA AATCATAAAC TGACTCTTGG TTTGATTTGG 540

AATATAATCC TCCACTGGCA GGTCAAAAAT GTAATGAAAA ATATCATGGC TGGATTGCAA 600

CAAACCAACA GTGAAAAGAT TCTCCTGAGC TGGGTCCGAC AATCAACTCG TAATTATCCA 660

CAGGTTAATG TAATCAACTT CACCACCAGC TGGTCTGATG GCCTGGCTTT GAATGCTCTC 720

ATCCATAGTC ATAGGCCAGA CCTATTTGAC TGGAATAGTG TGGTTTGCCA GCAGTCAGCC 780

ACACAACGAC TGGAACATGC ATTCAACATC GCCAGATATC AATTAGGCAT AGAGAAACTA 840

CTCGATCCTG AAGATGTTGA TACCACCTAT CCAGATAAGA AGTCCATCTT AATGTACATC 900

ACATCACTCT TCCAAGTTTT GCCTCAACAA GTGAGCATTG AAGCCATCCA GGAAGTGGAA 960

ATGTTGCCAA GGCCACCTAA AGTGACTAAA GAAGAACATT TTCAGTTACA TCATCAAATG 1020

CACTATTCTC AACAGATCAC GGTCAGTCTA GCACAGGGAT ATGAGAGAAC TTCTTCCCCT 1080

AAGCCTCGAT TCAAGAGCTA TGCCTACACA CAGGCTGCTT ATGTCACCAC CTCTGACCCT 1140

ACACGGAGCC CATTTCCTTC ACAGCATTTG GAAGCTCCTG AAGACAAGTC ATTTGGCAGT 1200

TCATTGATGG AGAGTGAAGT AAACCTGGAC CGTTATCAAA CAGCTTTAGA AGAAGTATTA 1260

TCGTGGCTTC TTTCTGCTGA GGACACATTG CAAGCACAAG GAGAGATTTC TAATGATGTG 1320

GAAGTGGTGA AAGACCAGTT TCATACTCAT GAGGGGTACA TGATGGATTT GACAGCCCAT 1380

CAGGGCCGGG TTGGTAATAT TCTACAATTG GGAAGTAAGC TGATTGGAAC AGGAAAATTA 1440

TCAGAAGATG AAGAAACTGA AGTACAAGAG CAGATGAATC TCCTAAATTC AAGATGGGAA 1500

TGCCTCAGGG TAGCTAGCAT GGAAAAACAA AGCAATTTAC ATAGAGTTTT AATGGATCTC 1560

CAGAATCAGA AACTGAAAGA GTTGAATGAC TGGCTAACAA AAACAGAAGA AAGAACAAGG 1620

AAAATGGAGG AAGAGCCTCT TGGACCTGAT CTTGAAGACC TAAAACGCCA AGTACAACAA 1680

CATAAGGTGC TTCAAGAAGA TCTAGAACAA GAACAAGTCA GGGTCAATTC TCTCACTCAC 1740

ATGGTGGTGG TAGTTGATGA ATCTAGTGGA GATCACGCAA CTGCTGCTTT GGAAGAACAA 1800

CTTAAGGTAT TGGGAGATCG ATGGGCAAAC ATCTGTAGAT GGACAGAAGA CCGCTGGGTT 1860

CTTTTACAAG ACATCCTTCT CAAATGGCAA CGTCTTACTG AAGAACAGTG CCTTTTTAGT 1920

GCATGGCTTT CAGAAAAAGA AGATGCAGTG AACAAGATTC ACACAACTGG CTTTAAAGAT 1980

CAAAATGAAA TGTTATCAAG TCTCGAGAAA GTCAAGGCAC TTCGAGGAGA AATTGCGCCT 2040

CTGAAAGAGA ACGTGAGCCA CGTCAATGAC CTTGCTCGCC AGCTTACCAC TTTGGGCATT 2100

CAGCTCTCAC CGTATAACCT CAGCACTCTG GAAGACCTGA ACACCAGATG GAAGCTTCTG 2160

CAGGTGGCCG TCGAGGACCG AGTCAGGCAG CTGCATGAAG CCCACAGGGA CTTTGGTCCA 2220

GCATCTCAGC ACTTTCTTTC CACGTCTGTC CAGGGTCCCT GGGAGAGAGC CATCTCGCCA 2280

AACAAAGTGC CCTACTATAT CAACCACGAG ACTCAAACAA CTTGCTGGGA CCATCCCAAA 2340

ATGACAGAGC TCTACCAGTC TTTAGCTGAC CTGAATAATG TCAGATTCTC AGCTTATAGG 2400

ACTGCCATGA AACTCCGAAG ACTGCAGAAG GCCCTTTGCT TGGATCTCTT GAGCCTGTCA 2460

GCTGCATGTG ATGCCTTGGA CCAGCACAAC CTCAAGCAAA ATGACCAGCC CATGGATATC 2520

CTGCAGATTA TTAATTGTTT GACCACTATT TATGACCGCC TGGAGCAAGA GCACAACAAT 2580

TTGGTCAACG TCCCTCTCTG CGTGGATATG TGTCTGAACT GGCTGCTGAA TGTTTATGAT 2640

ACGGGACGAA CAGGGAGGAT CCGTGTCCTG TCTTTTAAAA CTGGCATCAT TTCCCTGTGT 2700

AAAGCACATT TGGAAGACAA GTACAGATAC CTTTTCAAGC AAGTGGCAAG TTCAACAGGA 2760

TTTTGTGACC AGCGCAGGCT GGGCCTCCTT CTGCATGATT CTATCCAAAT TCCAAGACAG 2820

TTGGGTGAAG TTGCATCCTT TGGGGGCAGT AACATTGAGC CAAGTGTCCG GAGCTGCTTC 2880

CAATTTGCTA ATAATAAGCC AGAGATCGAA GCGGCCCTCT TCCTAGACTG GATGAGACTG 2940

GAACCCCAGT CCATGGTGTG GCTGCCCGTC CTGCACAGAG TGGCTGCTGC AGAAACTGCC 3000

AAGCATCAGG CCAAATGTAA CATCTGCAAA GAGTGTCCAA TCATTGGATT CAGGTACAGG 3060

AGTCTAAAGC ACTTTAATTA TGACATCTGC CAAAGCTGCT TTTTTTCTGG TCGAGTTGCA 3120

AAAGGCCATA AAATGCACTA TCCCATGGTG GAATATTGCA CTCCGACTAC ATCAGGAGAA 3180

GATGTTCGAG ACTTTGCCAA GGTACTAAAA AACAAATTTC GAACCAAAAG GTATTTTGCG 3240

AAGCATCCCC GAATGGGCTA CCTGCCAGTG CAGACTGTCT TAGAGGGGGA CAACATGGAA 3300

ACTCCCGTTA CTCTGATCAA CTTCTGGCCA GTAGATTCTG CGCCTGCCTC GTCCCCTCAG 3360

CTTTCACACG ATGATACTCA TTCACGCATT GAACATTATG CTAGCAGGCT AGCAGAAATG 3420

GAAAACAGCA ATGGATCTTA TCTAAATGAT AGCATCTCTC CTAATGAGAG CATAGATGAT 3480

GAACATTTGT TAATCCAGCA TTACTGCCAA AGTTTGAACC AGGACTCCCC CCTGAGCCAG 3540

CCTCGTAGTC CTGCCCAGAT CTTGATTTCC TTAGAGAGTG AGGAAAGAGG GGAGCTAGAG 3600

AGAATCCTAG CAGATCTTGA GGAAGAAAAC AGGAATCTGC AAGCAGAATA TGACCGTCTA 3660

AAGCAGCAGC ACGAACATAA AGGCCTGTCC CCACTGCCGT CCCCTCCTGA AATGATGCCC 3720

ACCTCTCCCC AGAGTCCCCG GGATGCTGAG CTCATTGCTG AGGCCAAGCT ACTGCGTCAA 3780

CACAAAGGCC GCCTGGAAGC CAGGATGCAA ATCCTGGAAG ACCACAATAA ACAGCTGGAG 3840

TCACAGTTAC ACAGGCTAAG GCAGCTGCTG GAGCAACCCC AGGCAGAGGC CAAAGTGAAT 3900

GGCACAACGG TGTCCTCTCC TTCTACCTCT CTACAGAGGT CCGACAGCAG TCAGCCTATG 3960

CTGCTCCGAG TGGTTGGCAG TCAAACTTCG GACTCCATGG GTGAGGAAGA TCTTCTCAGT 4020

CCTCCCCAGG ACACAAGCAC AGGGTTAGAG GAGGTGATGG AGCAACTCAA CAACTCCTTC 4080

CCTAGTTCAA GAGGAAGAAA TACCCCTGGA AAGCCAATGA GAGAGGACAC AATGTAGGAA 4140

GTCTTTTCCA CATGGCAGAT GATTTGGGCA GAGCGATGGA GTCCTTAGTA TCAGTCATGA 4200

CAGATGAAGA AGGAGCAGAA TAAATGTTTT ACAACTCCTG ATTCCCGCAT GGTTTTTATA 4260

ATATTCATAC AACAAAGAGG ATTAGACAGT AAGAGTTTAC AAGAAATAAA TCTATATTTT 4320

TGTGAAGGGT AGTGGTATTA TACTGTAGAT TTCAGTAGTT TCTAAGTCTG TTATTGTTTT 4380

GTTGGGGATC CTCTAGAGTC GA 4402

Sequence Number: 6

Length of sequence: 1,310

Form of sequence: amino acid

Topology: straight chain

Kind of sequence: protein

Arrangement

Met Leu Trp Trp Glu Glu Val Glu Asp Cys Tyr Glu Arg Glu Asp 15

Val Gln Lys Lys Thr Phe Thr Lys Trp Val Asn Ala Gln Phe Ser 30

Lys Phe Gly Lys Gln His Ile Glu Asn Leu Phe Ser Asp Leu Gln 45

Asp Gly Arg Arg Leu Leu Asp Leu Leu Glu Gly Leu Thr Gly Gln 60

Lys Leu Pro Lys Glu Lys Gly Ser Thr Arg Val His Ala Leu Asn 75

Asn Val Asn Lys Ala Leu Arg Val Leu Gln Asn Asn Asn Val Asp 90

Leu Val Asn Ile Gly Ser Thr Asp Ile Val Asp Gly Asn His Lys 105

Leu Thr Leu Gly Leu Ile Trp Asn Ile Ile Leu His Trp Gln Val 120

Lys Asn Val Met Lys Asn Ile Met Ala Gly Leu Gln Gln Thr Asn 135

Ser Glu Lys Ile Leu Leu Ser Trp Val Arg Gln Ser Thr Arg Asn 150

Tyr Pro Gln Val Asn Val Ile Asn Phe Thr Thr Ser Trp Ser Asp 165

Gly Leu Ala Leu Asn Ala Leu Ile His Ser His Arg Pro Asp Leu 180

Phe Asp Trp Asn Ser Val Val Cys Gln Gln Ser Ala Thr Gln Arg 195

Leu Glu His Ala Phe Asn Ile Ala Arg Tyr Gln Leu Gly Ile Glu 210

Lys Leu Leu Asp Pro Glu Asp Val Asp Thr Thr Tyr Pro Asp Lys 225

Lys Ser Ile Leu Met Tyr Ile Thr Ser Leu Phe Gln Val Leu Pro 240

Gln Gln Val Ser Ile Glu Ala Ile Gln Glu Val Glu Met Leu Pro 255

Arg Pro Pro Lys Val Thr Lys Glu Glu His Phe Gln Leu His His 270

Gln Met His Tyr Ser Gln Gln Ile Thr Val Ser Leu Ala Gln Gly 285

Tyr Glu Arg Thr Ser Ser Pro Lys Pro Arg Phe Lys Ser Tyr Ala 300

Tyr Thr Gln Ala Ala Tyr Val Thr Thr Ser Asp Pro Thr Arg Ser 315

Pro Phe Pro Ser Gln His Leu Glu Ala Pro Glu Asp Lys Ser Phe 330

Gly Ser Ser Leu Met Glu Ser Glu Val Asn Leu Asp Arg Tyr Gln 345

Thr Ala Leu Glu Glu Val Leu Ser Trp Leu Leu Ser Ala Glu Asp 360

Thr Leu Gln Ala Gln Gly Glu Ile Ser Asn Asp Val Glu Val Val 375

Lys Asp Gln Phe His Thr His Glu Gly Tyr Met Met Asp Leu Thr 390

Ala His Gln Gly Arg Val Gly Asn Ile Leu Gln Leu Gly Ser Lys 405

Leu Ile Gly Thr Gly Lys Leu Ser Glu Asp Glu Glu Thr Glu Val 420

Gln Glu Gln Met Asn Leu Leu Asn Ser Arg Trp Glu Cys Leu Arg 435

Val Ala Ser Met Glu Lys Gln Ser Asn Leu His Arg Val Leu Met 450

Asp Leu Gln Asn Gln Lys Leu Lys Glu Leu Asn Asp Trp Leu Thr 465

Lys Thr Glu Glu Arg Thr Arg Lys Met Glu Glu Glu Pro Leu Gly 480

Pro Asp Leu Glu Asp Leu Lys Arg Gln Val Gln Gln His Lys Val 495

Leu Gln Glu Asp Leu Glu Gln Glu Gln Val Arg Val Asn Ser Leu 510

Thr His Met Val Val Val Val Asp Glu Ser Ser Gly Asp His Ala 525

Thr Ala Ala Leu Glu Glu Gln Leu Lys Val Leu Gly Asp Arg Trp 540

Ala Asn Ile Cys Arg Trp Thr Glu Asp Arg Trp Val Leu Leu Gln 555

Asp Ile Leu Leu Lys Trp Gln Arg Leu Thr Glu Glu Gln Cys Leu 570

Phe Ser Ala Trp Leu Ser Glu Lys Glu Asp Ala Val Asn Lys Ile 585

His Thr Thr Gly Phe Lys Asp Gln Asn Glu Met Leu Ser Ser Leu 600

Glu Lys Val Lys Ala Leu Arg Gly Glu Ile Ala Pro Leu Lys Glu 615

Asn Val Ser His Val Asn Asp Leu Ala Arg Gln Leu Thr Thr Leu 630

Gly Ile Gln Leu Ser Pro Tyr Asn Leu Ser Thr Leu Glu Asp Leu 645

Asn Thr Arg Trp Lys Leu Leu Gln Val Ala Val Glu Asp Arg Val 660

Arg Gln Leu His Glu Ala His Arg Asp Phe Gly Pro Ala Ser Gln 675

His Phe Leu Ser Thr Ser Val Gln Gly Pro Trp Glu Arg Ala Ile 690

Ser Pro Asn Lys Val Pro Tyr Tyr Ile Asn His Glu Thr Gln Thr 705

Thr Cys Trp Asp His Pro Lys Met Thr Glu Leu Tyr Gln Ser Leu 720

Ala Asp Leu Asn Asn Val Arg Phe Ser Ala Tyr Arg Thr Ala Met 735

Lys Leu Arg Arg Leu Gln Lys Ala Leu Cys Leu Asp Leu Leu Ser 750

Leu Ser Ala Ala Cys Asp Ala Leu Asp Gln His Asn Leu Lys Gln 765

Asn Asp Gln Pro Met Asp Ile Leu Gln Ile Ile Asn Cys Leu Thr 780

Thr Ile Tyr Asp Arg Leu Glu Gln Glu His Asn Asn Leu Val Asn 795

Val Pro Leu Cys Val Asp Met Cys Leu Asn Trp Leu Leu Asn Val 810

Tyr Asp Thr Gly Arg Thr Gly Arg Ile Arg Val Leu Ser Phe Lys 825

Thr Gly Ile Ile Ser Leu Cys Lys Ala His Leu Glu Asp Lys Tyr 840

Arg Tyr Leu Phe Lys Gln Val Ala Ser Ser Thr Gly Phe Cys Asp 855

Gln Arg Arg Leu Gly Leu Leu Leu His Asp Ser Ile Gln Ile Pro 870

Arg Gln Leu Gly Glu Val Ala Ser Phe Gly Gly Ser Asn Ile Glu 885

Pro Ser Val Arg Ser Cys Phe Gln Phe Ala Asn Asn Lys Pro Glu 900

Ile Glu Ala Ala Leu Phe Leu Asp Trp Met Arg Leu Glu Pro Gln 915

Ser Met Val Trp Leu Pro Val Leu His Arg Val Ala Ala Ala Glu 930

Thr Ala Lys His Gln Ala Lys Cys Asn Ile Cys Lys Glu Cys Pro 945

Ile Ile Gly Phe Arg Tyr Arg Ser Leu Lys His Phe Asn Tyr Asp 960

Ile Cys Gln Ser Cys Phe Phe Ser Gly Arg Val Ala Lys Gly His 975

Lys Met His Tyr Pro Met Val Glu Tyr Cys Thr Pro Thr Thr Ser 990

Gly Glu Asp Val Arg Asp Phe Ala Lys Val Leu Lys Asn Lys Phe 1005

Arg Thr Lys Arg Tyr Phe Ala Lys His Pro Arg Met Gly Tyr Leu 1020

Pro Val Gln Thr Val Leu Glu Gly Asp Asn Met Glu Thr Pro Val 1035

Thr Leu Ile Asn Phe Trp Pro Val Asp Ser Ala Pro Ala Ser Ser 1050

Pro Gln Leu Ser His Asp Asp Thr His Ser Arg Ile Glu His Tyr 1065

Ala Ser Arg Leu Ala Glu Met Glu Asn Ser Asn Gly Ser Tyr Leu 1080

Asn Asp Ser Ile Ser Pro Asn Glu Ser Ile Asp Asp Glu His Leu 1095

Leu Ile Gln His Tyr Cys Gln Ser Leu Asn Gln Asp Ser Pro Leu 1110

Ser Gln Pro Arg Ser Pro Ala Gln Ile Leu Ile Ser Leu Glu Ser 1125

Glu Glu Arg Gly Glu Leu Glu Arg Ile Leu Ala Asp Leu Glu Glu 1140

Glu Asn Arg Asn Leu Gln Ala Glu Tyr Asp Arg Leu Lys Gln Gln 1155

His Glu His Lys Gly Leu Ser Pro Leu Pro Ser Pro Pro Glu Met 1170

Met Pro Thr Ser Pro Gln Ser Pro Arg Asp Ala Glu Leu Ile Ala 1185

Glu Ala Lys Leu Leu Arg Gln His Lys Gly Arg Leu Glu Ala Arg 1200

Met Gln Ile Leu Glu Asp His Asn Lys Gln Leu Glu Ser Gln Leu 1215

His Arg Leu Arg Gln Leu Leu Glu Gln Pro Gln Ala Glu Ala Lys 1230

Val Asn Gly Thr Thr Val Ser Ser Pro Ser Thr Ser Leu Gln Arg 1245

Ser Asp Ser Ser Gln Pro Met Leu Leu Arg Val Val Gly Ser Gln 1260

Thr Ser Asp Ser Met Gly Glu Glu Asp Leu Leu Ser Pro Pro Gln 1275

Asp Thr Ser Thr Gly Leu Glu Glu Val Met Glu Gln Leu Asn Asn 1290

Ser Phe Pro Ser Ser Arg Gly Arg Asn Thr Pro Gly Lys Pro Met 1305

Arg Glu Asp Thr Met 1310

Sequence Number: 7

Length of sequence: 4075

Form of sequence: nucleic acid

Number of strands: Both morphological form (both)

Topology: straight chain

Kind of sequence: Feature: active-site of cDNA to mRNA arrangement

Arrangement

CGGCCGCTCT AGAGGATCCC CGGGTACCGA GCTCGAATTC CGGAACTCCC GGAGAAAAAC 60

GAATAGGAAA AACTGAAGTG TTACTTTTTT TAAAGCTGCT GAAGTTTGTT GGTTTCTCAT 120

TGTTTTTAAG CCTACTGGAG CAATAAAGTT TGAAGAACTT TTACCAGGTT TTTTTTATCG 180

CTGCCTTGAT ATACACTTTT CAAAATGCTT TGGTGGGAAG AAGTAGAGGA CTGTTATGAA 240

AGAGAAGATG TTCAAAAGAA AACATTCACA AAATGGGTAA ATGCACAATT TTCTAAGTTT 300

GGGAAGCAGC ATATTGAGAA CCTCTTCAGT GACCTACAGG ATGGGAGGCG CCTCCTAGAC 360

CTCCTCGAAG GCCTGACAGG GCAAAAACTG CCAAAAGAAA AAGGATCCAC AAGAGTTCAT 420

GCCCTGAACA ATGTCAACAA GGCACTGCGG GTTTTGCAGA ACAATAATGT TGATTTAGTG 480

AATATTGGAA GTACTGACAT CGTAGATGGA AATCATAAAC TGACTCTTGG TTTGATTTGG 540

AATATAATCC TCCACTGGCA GGTCAAAAAT GTAATGAAAA ATATCATGGC TGGATTGCAA 600

CAAACCAACA GTGAAAAGAT TCTCCTGAGC TGGGTCCGAC AATCAACTCG TAATTATCCA 660

CAGGTTAATG TAATCAACTT CACCACCAGC TGGTCTGATG GCCTGGCTTT GAATGCTCTC 720

ATCCATAGTC ATAGGCCAGA CCTATTTGAC TGGAATAGTG TGGTTTGCCA GCAGTCAGCC 780

ACACAACGAC TGGAACATGC ATTCAACATC GCCAGATATC AATTAGGCAT AGAGAAACTA 840

CTCGATCCTG AAGATGTTGA TACCACCTAT CCAGATAAGA AGTCCATCTT AATGTACATC 900

ACATCACTCT TCCAAGTTTT GCCTCAACAA GTGAGCATTG AAGCCATCCA GGAAGTGGAA 960

ATGTTGCCAA GGCCACCTAA AGTGACTAAA GAAGAACATT TTCAGTTACA TCATCAAATG 1020

CACTATTCTC AACAGATCAC GGTCAGTCTA GCACAGGGAT ATGAGAGAAC TTCTTCCCCT 1080

AAGCCTCGAT TCAAGAGCTA TGCCTACACA CAGGCTGCTT ATGTCACCAC CTCTGACCCT 1140

ACACGGAGCC CATTTCCTTC ACAGCATTTG GAAGCTCCTG AAGACAAGTC ATTTGGCAGT 1200

TCATTGATGG AGAGTGAAGT AAACCTGGAC CGTTATCAAA CAGCTTTAGA AGAAGTATTA 1260

TCGTGGCTTC TTTCTGCTGA GGACACATTG CAAGCACAAG GAGAGATTTC TAATGATGTG 1320

GAAGTGGTGA AAGACCAGTT TCATACTCAT GAGGGGTACA TGATGGATTT GACAGCCCAT 1380

CAGGGCCGGG TTGGTAATAT TCTACAATTG GGAAGTAAGC TGATTGGAAC AGGAAAATTA 1440

TCAGAAGATG AAGAAACTGA AGTACAAGAG CAGATGAATC TCCTAAATTC AAGATGGGAA 1500

TGCCTCAGGG TAGCTAGCAT GGAAAAACAA AGCAATTTAC ATAGAGTTTT AATGGATCTC 1560

CAGAATCAGA AACTGAAAGA GTTGAATGAC TGGCTAACAA AAACAGAAGA AAGAACAAGG 1620

AAAATGGAGG AAGAGCCTCT TGGACCTGAT CTTGAAGACC TAAAACGCCA AGTACAACAA 1680

CATAAGGTGC TTCAAGAAGA TCTAGAACAA GAACAAGTCA GGGTCAATTC TCTCACTCAC 1740

ATGGTGGTGG TAGTTGATGA ATCTAGTGGA GATCACGCAA CTGCTGCTTT GGAAGAACAA 1800

CTTAAGGTAT TGAACACCAG ATGGAAGCTT CTGCAGGTGG CCGTCGAGGA CCGAGTCAGG 1860

CAGCTGCATG AAGCCCACAG GGACTTTGGT CCAGCATCTC AGCACTTTCT TTCCACGTCT 1920

GTCCAGGGTC CCTGGGAGAG AGCCATCTCG CCAAACAAAG TGCCCTACTA TATCAACCAC 1980

GAGACTCAAA CAACTTGCTG GGACCATCCC AAAATGACAG AGCTCTACCA GTCTTTAGCT 2040

GACCTGAATA ATGTCAGATT CTCAGCTTAT AGGACTGCCA TGAAACTCCG AAGACTGCAG 2100

AAGGCCCTTT GCTTGGATCT CTTGAGCCTG TCAGCTGCAT GTGATGCCTT GGACCAGCAC 2160

AACCTCAAGC AAAATGACCA GCCCATGGAT ATCCTGCAGA TTATTAATTG TTTGACCACT 2220

ATTTATGACC GCCTGGAGCA AGAGCACAAC AATTTGGTCA ACGTCCCTCT CTGCGTGGAT 2280

ATGTGTCTGA ACTGGCTGCT GAATGTTTAT GATACGGGAC GAACAGGGAG GATCCGTGTC 2340

CTGTCTTTTA AAACTGGCAT CATTTCCCTG TGTAAAGCAC ATTTGGAAGA CAAGTACAGA 2400

TACCTTTTCA AGCAAGTGGC AAGTTCAACA GGATTTTGTG ACCAGCGCAG GCTGGGCCTC 2460

CTTCTGCATG ATTCTATCCA AATTCCAAGA CAGTTGGGTG AAGTTGCATC CTTTGGGGGC 2520

AGTAACATTG AGCCAAGTGT CCGGAGCTGC TTCCAATTTG CTAATAATAA GCCAGAGATC 2580

GAAGCGGCCC TCTTCCTAGA CTGGATGAGA CTGGAACCCC AGTCCATGGT GTGGCTGCCC 2640

GTCCTGCACA GAGTGGCTGC TGCAGAAACT GCCAAGCATC AGGCCAAATG TAACATCTGC 2700

AAAGAGTGTC CAATCATTGG ATTCAGGTAC AGGAGTCTAA AGCACTTTAA TTATGACATC 2760

TGCCAAAGCT GCTTTTTTTC TGGTCGAGTT GCAAAAGGCC ATAAAATGCA CTATCCCATG 2820

GTGGAATATT GCACTCCGAC TACATCAGGA GAAGATGTTC GAGACTTTGC CAAGGTACTA 2880

AAAAACAAAT TTCGAACCAA AAGGTATTTT GCGAAGCATC CCCGAATGGG CTACCTGCCA 2940

GTGCAGACTG TCTTAGAGGG GGACAACATG GAAACTCCCG TTACTCTGAT CAACTTCTGG 3000

CCAGTAGATT CTGCGCCTGC CTCGTCCCCT CAGCTTTCAC ACGATGATAC TCATTCACGC 3060

ATTGAACATT ATGCTAGCAG GCTAGCAGAA ATGGAAAACA GCAATGGATC TTATCTAAAT 3120

GATAGCATCT CTCCTAATGA GAGCATAGAT GATGAACATT TGTTAATCCA GCATTACTGC 3180

CAAAGTTTGA ACCAGGACTC CCCCCTGAGC CAGCCTCGTA GTCCTGCCCA GATCTTGATT 3240

TCCTTAGAGA GTGAGGAAAG AGGGGAGCTA GAGAGAATCC TAGCAGATCT TGAGGAAGAA 3300

AACAGGAATC TGCAAGCAGA ATATGACCGT CTAAAGCAGC AGCACGAACA TAAAGGCCTG 3360

TCCCCACTGC CGTCCCCTCC TGAAATGATG CCCACCTCTC CCCAGAGTCC CCGGGATGCT 3420

GAGCTCATTG CTGAGGCCAA GCTACTGCGT CAACACAAAG GCCGCCTGGA AGCCAGGATG 3480

CAAATCCTGG AAGACCACAA TAAACAGCTG GAGTCACAGT TACACAGGCT AAGGCAGCTG 3540

CTGGAGCAAC CCCAGGCAGA GGCCAAAGTG AATGGCACAA CGGTGTCCTC TCCTTCTACC 3600

TCTCTACAGA GGTCCGACAG CAGTCAGCCT ATGCTGCTCC GAGTGGTTGG CAGTCAAACT 3660

TCGGACTCCA TGGGTGAGGA AGATCTTCTC AGTCCTCCCC AGGACACAAG CACAGGGTTA 3720

GAGGAGGTGA TGGAGCAACT CAACAACTCC TTCCCTAGTT CAAGAGGAAG AAATACCCCT 3780

GGAAAGCCAA TGAGAGAGGA CACAATGTAG GAAGTCTTTT CCACATGGCA GATGATTTGG 3840

GCAGAGCGAT GGAGTCCTTA GTATCAGTCA TGACAGATGA AGAAGGAGCA GAATAAATGT 3900

TTTACAACTC CTGATTCCCG CATGGTTTTT ATAATATTCA TACAACAAAG AGGATTAGAC 3960

AGTAAGAGTT TACAAGAAAT AAATCTATAT TTTTGTGAAG GGTAGTGGTA TTATACTGTA 4020

GATTTCAGTA GTTTCTAAGT CTGTTATTGT TTTGTTGGGG ATCCTCTAGA GTCGA 4075

Sequence Number: 8

Length of sequence: 1201

Form of sequence: amino acid
Topology: straight chain
Kind of sequence: protein
Arrangement

Met Leu Trp Trp Glu Glu Val Glu Asp Cys Tyr Glu Arg Glu Asp 15

Val Gln Lys Lys Thr Phe Thr Lys Trp Val Asn Ala Gln Phe Ser 30

Lys Phe Gly Lys Gln His Ile Glu Asn Leu Phe Ser Asp Leu Gln 45

Asp Gly Arg Arg Leu Leu Asp Leu Leu Glu Gly Leu Thr Gly Gln 60

Lys Leu Pro Lys Glu Lys Gly Ser Thr Arg Val His Ala Leu Asn 75

Asn Val Asn Lys Ala Leu Arg Val Leu Gln Asn Asn Asn Val Asp 90

Leu Val Asn Ile Gly Ser Thr Asp Ile Val Asp Gly Asn His Lys 105

Leu Thr Leu Gly Leu Ile Trp Asn Ile Ile Leu His Trp Gln Val 120

Lys Asn Val Met Lys Asn Ile Met Ala Gly Leu Gln Gln Thr Asn 135

Ser Glu Lys Ile Leu Leu Ser Trp Val Arg Gln Ser Thr Arg Asn 150

Tyr Pro Gln Val Asn Val Ile Asn Phe Thr Thr Ser Trp Ser Asp 165

Gly Leu Ala Leu Asn Ala Leu Ile His Ser His Arg Pro Asp Leu 180

Phe Asp Trp Asn Ser Val Val Cys Gln Gln Ser Ala Thr Gln Arg 195

Leu Glu His Ala Phe Asn Ile Ala Arg Tyr Gln Leu Gly Ile Glu 210

Lys Leu Leu Asp Pro Glu Asp Val Asp Thr Thr Tyr Pro Asp Lys 225

Lys Ser Ile Leu Met Tyr Ile Thr Ser Leu Phe Gln Val Leu Pro 240

Gln Gln Val Ser Ile Glu Ala Ile Gln Glu Val Glu Met Leu Pro 255

Arg Pro Pro Lys Val Thr Lys Glu Glu His Phe Gln Leu His His 270

Gln Met His Tyr Ser Gln Gln Ile Thr Val Ser Leu Ala Gln Gly 285

Tyr Glu Arg Thr Ser Ser Pro Lys Pro Arg Phe Lys Ser Tyr Ala 300

Tyr Thr Gln Ala Ala Tyr Val Thr Thr Ser Asp Pro Thr Arg Ser 315

Pro Phe Pro Ser Gln His Leu Glu Ala Pro Glu Asp Lys Ser Phe 330

Gly Ser Ser Leu Met Glu Ser Glu Val Asn Leu Asp Arg Tyr Gln 345

Thr Ala Leu Glu Glu Val Leu Ser Trp Leu Leu Ser Ala Glu Asp 360

Thr Leu Gln Ala Gln Gly Glu Ile Ser Asn Asp Val Glu Val Val 375

Lys Asp Gln Phe His Thr His Glu Gly Tyr Met Met Asp Leu Thr 390

Ala His Gln Gly Arg Val Gly Asn Ile Leu Gln Leu Gly Ser Lys 405

Leu Ile Gly Thr Gly Lys Leu Ser Glu Asp Glu Glu Thr Glu Val 420

Gln Glu Gln Met Asn Leu Leu Asn Ser Arg Trp Glu Cys Leu Arg 435

Val Ala Ser Met Glu Lys Gln Ser Asn Leu His Arg Val Leu Met 450

Asp Leu Gln Asn Gln Lys Leu Lys Glu Leu Asn Asp Trp Leu Thr 465

Lys Thr Glu Glu Arg Thr Arg Lys Met Glu Glu Glu Pro Leu Gly 480

Pro Asp Leu Glu Asp Leu Lys Arg Gln Val Gln Gln His Lys Val 495

Leu Gln Glu Asp Leu Glu Gln Glu Gln Val Arg Val Asn Ser Leu 510

Thr His Met Val Val Val Val Asp Glu Ser Ser Gly Asp His Ala 525

Thr Ala Ala Leu Glu Glu Gln Leu Lys Val Leu Asn Thr Arg Trp 540

Lys Leu Leu Gln Val Ala Val Glu Asp Arg Val Arg Gln Leu His 555

Glu Ala His Arg Asp Phe Gly Pro Ala Ser Gln His Phe Leu Ser 570

Thr Ser Val Gln Gly Pro Trp Glu Arg Ala Ile Ser Pro Asn Lys 585

Val Pro Tyr Tyr Ile Asn His Glu Thr Gln Thr Thr Cys Trp Asp 600

His Pro Lys Met Thr Glu Leu Tyr Gln Ser Leu Ala Asp Leu Asn 615

Asn Val Arg Phe Ser Ala Tyr Arg Thr Ala Met Lys Leu Arg Arg 630

Leu Gln Lys Ala Leu Cys Leu Asp Leu Leu Ser Leu Ser Ala Ala 645

Cys Asp Ala Leu Asp Gln His Asn Leu Lys Gln Asn Asp Gln Pro 660

Met Asp Ile Leu Gln Ile Ile Asn Cys Leu Thr Thr Ile Tyr Asp 675

Arg Leu Glu Gln Glu His Asn Asn Leu Val Asn Val Pro Leu Cys 690

Val Asp Met Cys Leu Asn Trp Leu Leu Asn Val Tyr Asp Thr Gly 705

Arg Thr Gly Arg Ile Arg Val Leu Ser Phe Lys Thr Gly Ile Ile 720

Ser Leu Cys Lys Ala His Leu Glu Asp Lys Tyr Arg Tyr Leu Phe 735

Lys Gln Val Ala Ser Ser Thr Gly Phe Cys Asp Gln Arg Arg Leu 750

Gly Leu Leu Leu His Asp Ser Ile Gln Ile Pro Arg Gln Leu Gly 765

Glu Val Ala Ser Phe Gly Gly Ser Asn Ile Glu Pro Ser Val Arg 780

Ser Cys Phe Gln Phe Ala Asn Asn Lys Pro Glu Ile Glu Ala Ala 795

Leu Phe Leu Asp Trp Met Arg Leu Glu Pro Gln Ser Met Val Trp 810

Leu Pro Val Leu His Arg Val Ala Ala Ala Glu Thr Ala Lys His 825

Gln Ala Lys Cys Asn Ile Cys Lys Glu Cys Pro Ile Ile Gly Phe 840

Arg Tyr Arg Ser Leu Lys His Phe Asn Tyr Asp Ile Cys Gln Ser 855

Cys Phe Phe Ser Gly Arg Val Ala Lys Gly His Lys Met His Tyr 870

Pro Met Val Glu Tyr Cys Thr Pro Thr Thr Ser Gly Glu Asp Val 885

Arg Asp Phe Ala Lys Val Leu Lys Asn Lys Phe Arg Thr Lys Arg 900

Tyr Phe Ala Lys His Pro Arg Met Gly Tyr Leu Pro Val Gln Thr 915

Val Leu Glu Gly Asp Asn Met Glu Thr Pro Val Thr Leu Ile Asn 930

Phe Trp Pro Val Asp Ser Ala Pro Ala Ser Ser Pro Gln Leu Ser 945

His Asp Asp Thr His Ser Arg Ile Glu His Tyr Ala Ser Arg Leu 960

Ala Glu Met Glu Asn Ser Asn Gly Ser Tyr Leu Asn Asp Ser Ile 975

Ser Pro Asn Glu Ser Ile Asp Asp Glu His Leu Leu Ile Gln His 990

Tyr Cys Gln Ser Leu Asn Gln Asp Ser Pro Leu Ser Gln Pro Arg 1005

Ser Pro Ala Gln Ile Leu Ile Ser Leu Glu Ser Glu Glu Arg Gly 1020

Glu Leu Glu Arg Ile Leu Ala Asp Leu Glu Glu Glu Asn Arg Asn 1035

Leu Gln Ala Glu Tyr Asp Arg Leu Lys Gln Gln His Glu His Lys 1050

Gly Leu Ser Pro Leu Pro Ser Pro Pro Glu Met Met Pro Thr Ser 1065

Pro Gln Ser Pro Arg Asp Ala Glu Leu Ile Ala Glu Ala Lys Leu 1080

Leu Arg Gln His Lys Gly Arg Leu Glu Ala Arg Met Gln Ile Leu 1095

Glu Asp His Asn Lys Gln Leu Glu Ser Gln Leu His Arg Leu Arg 1110

Gln Leu Leu Glu Gln Pro Gln Ala Glu Ala Lys Val Asn Gly Thr 1125

Thr Val Ser Ser Pro Ser Thr Ser Leu Gln Arg Ser Asp Ser Ser 1140

Gln Pro Met Leu Leu Arg Val Val Gly Ser Gln Thr Ser Asp Ser 1155

Met Gly Glu Glu Asp Leu Leu Ser Pro Pro Gln Asp Thr Ser Thr 1170

Gly Leu Glu Glu Val Met Glu Gln Leu Asn Asn Ser Phe Pro Ser 1185

Ser Arg Gly Arg Asn Thr Pro Gly Lys Pro Met Arg Glu Asp Thr 1200

Met 1201

Sequence Number: 9

Length of sequence: 3,172

Form of sequence: nucleic acid

Number of strands: Both morphological form (both)

Topology: straight chain

Kind of sequence: Feature: active-site of cDNA to mRNA arrangement

Arrangement

CGGCCGCTCT AGAGGATCCC CGGGTACCGA GCTCGAATTC CGGAACTCCC GGAGAAAAAC 60

GAATAGGAAA AACTGAAGTG TTACTTTTTT TAAAGCTGCT GAAGTTTGTT GGTTTCTCAT 120

TGTTTTTAAG CCTACTGGAG CAATAAAGTT TGAAGAACTT TTACCAGGTT TTTTTTATCG 180

CTGCCTTGAT ATACACTTTT CAAAATGCTT TGGTGGGAAG AAGTAGAGGA CTGTTATGAA 240

AGAGAAGATG TTCAAAAGAA AACATTCACA AAATGGGTAA ATGCACAATT TTCTAAGTTT 300

GGGAAGCAGC ATATTGAGAA CCTCTTCAGT GACCTACAGG ATGGGAGGCG CCTCCTAGAC 360

CTCCTCGAAG GCCTGACAGG GCAAAAACTG CCAAAAGAAA AAGGATCCAC AAGAGTTCAT 420

GCCCTGAACA ATGTCAACAA GGCACTGCGG GTTTTGCAGA ACAATAATGT TGATTTAGTG 480

AATATTGGAA GTACTGACAT CGTAGATGGA AATCATAAAC TGACTCTTGG TTTGATTTGG 540

AATATAATCC TCCACTGGCA GGTCAAAAAT GTAATGAAAA ATATCATGGC TGGATTGCAA 600

CAAACCAACA GTGAAAAGAT TCTCCTGAGC TGGGTCCGAC AATCAACTCG TAATTATCCA 660

CAGGTTAATG TAATCAACTT CACCACCAGC TGGTCTGATG GCCTGGCTTT GAATGCTCTC 720

ATCCATAGTC ATAGGCCAGA CCTATTTGAC TGGAATAGTG TGGTTTGCCA GCAGTCAGCC 780

ACACAACGAC TGGAACATGC ATTCAACATC GCCAGATATC AATTAGGCAT AGAGAAACTA 840

CTCGATCCTG AAGATGTTGA TACCACCTAT CCAGATAAGA AGTCCATCTT AATGTACATC 900

ACATCACTCT TCCAAGTTTT GCCTCAACAA GTGAGCATTG AAGCCATCCA GGAAGTGGAA 960

ATGTTGCCAA GGCCACCTAA AGTGACTAAA GAAGAACATT TTCAGTTACA TCATCAAATG 1020

CACTATTCTC AACAGATCAC GGTCAGTCTA GCACAGGGAT ATGAGAGAAC TTCTTCCCCT 1080

AAGCCTCGAT TCAAGAGCTA TGCCTACACA CAGGCTGCTT ATGTCACCAC CTCTGACCCT 1140

ACACGGAGCC CATTTCCTTC ACAGCATTTG GAAGCTCCTG AAGACCGAAG ACTGCAGAAG 1200

GCCCTTTGCT TGGATCTCTT GAGCCTGTCA GCTGCATGTG ATGCCTTGGA CCAGCACAAC 1260

CTCAAGCAAA ATGACCAGCC CATGGATATC CTGCAGATTA TTAATTGTTT GACCACTATT 1320

TATGACCGCC TGGAGCAAGA GCACAACAAT TTGGTCAACG TCCCTCTCTG CGTGGATATG 1380

TGTCTGAACT GGCTGCTGAA TGTTTATGAT ACGGGACGAA CAGGGAGGAT CCGTGTCCTG 1440

TCTTTTAAAA CTGGCATCAT TTCCCTGTGT AAAGCACATT TGGAAGACAA GTACAGATAC 1500

CTTTTCAAGC AAGTGGCAAG TTCAACAGGA TTTTGTGACC AGCGCAGGCT GGGCCTCCTT 1560

CTGCATGATT CTATCCAAAT TCCAAGACAG TTGGGTGAAG TTGCATCCTT TGGGGGCAGT 1620

AACATTGAGC CAAGTGTCCG GAGCTGCTTC CAATTTGCTA ATAATAAGCC AGAGATCGAA 1680

GCGGCCCTCT TCCTAGACTG GATGAGACTG GAACCCCAGT CCATGGTGTG GCTGCCCGTC 1740

CTGCACAGAG TGGCTGCTGC AGAAACTGCC AAGCATCAGG CCAAATGTAA CATCTGCAAA 1800

GAGTGTCCAA TCATTGGATT CAGGTACAGG AGTCTAAAGC ACTTTAATTA TGACATCTGC 1860

CAAAGCTGCT TTTTTTCTGG TCGAGTTGCA AAAGGCCATA AAATGCACTA TCCCATGGTG 1920

GAATATTGCA CTCCGACTAC ATCAGGAGAA GATGTTCGAG ACTTTGCCAA GGTACTAAAA 1980

AACAAATTTC GAACCAAAAG GTATTTTGCG AAGCATCCCC GAATGGGCTA CCTGCCAGTG 2040

CAGACTGTCT TAGAGGGGGA CAACATGGAA ACTCCCGTTA CTCTGATCAA CTTCTGGCCA 2100

GTAGATTCTG CGCCTGCCTC GTCCCCTCAG CTTTCACACG ATGATACTCA TTCACGCATT 2160

GAACATTATG CTAGCAGGCT AGCAGAAATG GAAAACAGCA ATGGATCTTA TCTAAATGAT 2220

AGCATCTCTC CTAATGAGAG CATAGATGAT GAACATTTGT TAATCCAGCA TTACTGCCAA 2280

AGTTTGAACC AGGACTCCCC CCTGAGCCAG CCTCGTAGTC CTGCCCAGAT CTTGATTTCC 2340

TTAGAGAGTG AGGAAAGAGG GGAGCTAGAG AGAATCCTAG CAGATCTTGA GGAAGAAAAC 2400

AGGAATCTGC AAGCAGAATA TGACCGTCTA AAGCAGCAGC ACGAACATAA AGGCCTGTCC 2460

CCACTGCCGT CCCCTCCTGA AATGATGCCC ACCTCTCCCC AGAGTCCCCG GGATGCTGAG 2520

CTCATTGCTG AGGCCAAGCT ACTGCGTCAA CACAAAGGCC GCCTGGAAGC CAGGATGCAA 2580

ATCCTGGAAG ACCACAATAA ACAGCTGGAG TCACAGTTAC ACAGGCTAAG GCAGCTGCTG 2640

GAGCAACCCC AGGCAGAGGC CAAAGTGAAT GGCACAACGG TGTCCTCTCC TTCTACCTCT 2700

CTACAGAGGT CCGACAGCAG TCAGCCTATG CTGCTCCGAG TGGTTGGCAG TCAAACTTCG 2760

GACTCCATGG GTGAGGAAGA TCTTCTCAGT CCTCCCCAGG ACACAAGCAC AGGGTTAGAG 2820

GAGGTGATGG AGCAACTCAA CAACTCCTTC CCTAGTTCAA GAGGAAGAAA TACCCCTGGA 2980

AAGCCAATGA GAGAGGACAC AATGTAGGAA GTCTTTTCCA CATGGCAGAT GATTTGGGCA 2940

GAGCGATGGA GTCCTTAGTA TCAGTCATGA CAGATGAAGA AGGAGCAGAA TAAATGTTTT 3000

ACAACTCCTG ATTCCCGCAT GGTTTTTATA ATATTCATAC AACAAAGAGG ATTAGACAGT 3060

AAGAGTTTAC AAGAAATAAA TCTATATTTT TGTGAAGGGT AGTGGTATTA TACTGTAGAT 3120

TTCAGTAGTT TCTAAGTCTG TTATTGTTTT GTTGGGGATC CTCTAGAGTC GA 3172

Sequence Number: 10

Length of sequence: 900

Form of sequence: amino acid

Topology: straight chain

Kind of sequence: protein

Arrangement

Met Leu Trp Trp Glu Glu Val Glu Asp Cys Tyr Glu Arg Glu Asp 15

Val Gln Lys Lys Thr Phe Thr Lys Trp Val Asn Ala Gln Phe Ser 30

Lys Phe Gly Lys Gln His Ile Glu Asn Leu Phe Ser Asp Leu Gln 45

Asp Gly Arg Arg Leu Leu Asp Leu Leu Glu Gly Leu Thr Gly Gln 60

Lys Leu Pro Lys Glu Lys Gly Ser Thr Arg Val His Ala Leu Asn 75

Asn Val Asn Lys Ala Leu Arg Val Leu Gln Asn Asn Asn Val Asp 90

Leu Val Asn Ile Gly Ser Thr Asp Ile Val Asp Gly Asn His Lys 105

Leu Thr Leu Gly Leu Ile Trp Asn Ile Ile Leu His Trp Gln Val 120

Lys Asn Val Met Lys Asn Ile Met Ala Gly Leu Gln Gln Thr Asn 135

Ser Glu Lys Ile Leu Leu Ser Trp Val Arg Gln Ser Thr Arg Asn 150

Tyr Pro Gln Val Asn Val Ile Asn Phe Thr Thr Ser Trp Ser Asp 165

Gly Leu Ala Leu Asn Ala Leu Ile His Ser His Arg Pro Asp Leu 180

Phe Asp Trp Asn Ser Val Val Cys Gln Gln Ser Ala Thr Gln Arg 195

Leu Glu His Ala Phe AsnI le Ala Arg Tyr Gln Leu Gly Ile Glu 210

Lys Leu Leu Asp Pro Glu Asp Val Asp Thr Thr Tyr Pro Asp Lys 225

Lys Ser Ile Leu Met Tyr Ile Thr Ser Leu Phe Gln Val Leu Pro 240

Gln Gln Val Ser Ile Glu Ala Ile Gln Glu Val Glu Met Leu Pro 255

Arg Pro Pro Lys Val Thr Lys Glu Glu His Phe Gln Leu His His 270

Gln Met His Tyr Ser Gln Gln Ile Thr Val Ser Leu Ala Gln Gly 285

Tyr Glu Arg Thr Ser Ser Pro Lys Pro Arg Phe Lys Ser Tyr Ala 300

Tyr Thr Gln Ala Ala Tyr Val Thr Thr Ser Asp Pro Thr Arg Ser 315

Pro Phe Pro Ser Gln His Leu Glu Ala Pro Glu Asp Arg Arg Leu 330

Gln Lys Ala Leu Cys Leu Asp Leu Leu Ser Leu Ser Ala Ala Cys 345

Asp Ala Leu Asp Gln His Asn Leu Lys Gln Asn Asp Gln Pro Met 360

Asp Ile Leu Gln Ile Ile Asn Cys Leu Thr Thr Ile Tyr Asp Arg 375

Leu Glu Gln Glu His Asn Asn Leu Val Asn Val Pro Leu Cys Val 390

Asp Met Cys Leu Asn Trp Leu Leu Asn Val Tyr Asp Thr Gly Arg 405

Thr Gly Arg Ile Arg Val Leu Ser Phe Lys Thr Gly Ile Ile Ser 420

Leu Cys Lys Ala His Leu Glu Asp Lys Tyr Arg Tyr Leu Phe Lys 435

Gln Val Ala Ser Ser Thr Gly Phe Cys Asp Gln Arg Arg Leu Gly 450

Leu Leu Leu His Asp Ser Ile Gln Ile Pro Arg Gln Leu Gly Glu 465

Val Ala Ser Phe Gly Gly Ser Asn Ile Glu Pro Ser Val Arg Ser 480

Cys Phe Gln Phe Ala Asn Asn Lys Pro Glu Ile Glu Ala Ala Leu 495

Phe Leu Asp Trp Met Arg Leu Glu Pro Gln Ser Met Val Trp Leu 510

Pro Val Leu His Arg Val Ala Ala Ala Glu Thr Ala Lys His Gln 525

Ala Lys Cys Asn Ile Cys Lys Glu Cys Pro Ile Ile Gly Phe Arg 540
Tyr Arg Ser Leu Lys His Phe Asn Tyr Asp Ile Cys Gln Ser Cys 555
Phe Phe Ser Gly Arg Val Ala Lys Gly His Lys Met His Tyr Pro 570
Met Val Glu Tyr Cys Thr Pro Thr Thr Ser Gly Glu Asp Val Arg 585
Asp Phe Ala Lys Val Leu Lys Asn Lys Phe Arg Thr Lys Arg Tyr 600
Phe Ala Lys His Pro Arg Met Gly Tyr Leu Pro Val Gln Thr Val 615
Leu Glu Gly Asp Asn Met Glu Thr Pro Val Thr Leu Ile Asn Phe 630
Trp Pro Val Asp Ser Ala Pro Ala Ser Ser Pro Gln Leu Ser His 645
Asp Asp Thr His Ser Arg Ile Glu His Tyr Ala Ser Arg Leu Ala 660
Glu Met Glu Asn Ser Asn Gly Ser Tyr Leu Asn Asp Ser Ile Ser 675
Pro Asn Glu Ser Ile Asp Asp Glu His Leu Leu Ile Gln His Tyr 690
Cys Gln Ser Leu Asn Gln Asp Ser Pro Leu Ser Gln Pro Arg Ser 705
Pro Ala Gln Ile Leu Ile Ser Leu Glu Ser Glu Glu Arg Gly Glu 720
Leu Glu Arg Ile Leu Ala Asp Leu Glu Glu Glu Asn Arg Asn Leu 735
Gln Ala Glu Tyr Asp Arg Leu Lys Gln Gln His Glu His Lys Gly 750
Leu Ser Pro Leu Pro Ser Pro Pro Glu Met Met Pro Thr Ser Pro 765
Gln Ser Pro Arg Asp Ala Glu Leu Ile Ala Glu Ala Lys Leu Leu 780
Arg Gln His Lys Gly Arg Leu Glu Ala Arg Met Gln Ile Leu Glu 795
Asp His Asn Lys Gln Leu Glu Ser Gln Leu His Arg Leu Arg Gln 810
Leu Leu Glu Gln Pro Gln Ala Glu Ala Lys Val Asn Gly Thr Thr 825
Val Ser Ser Pro Ser Thr Ser Leu Gln Arg Ser Asp Ser Ser Gln 840
Pro Met Leu Leu Arg Val Val Gly Ser Gln Thr Ser Asp Ser Met 855
Gly Glu Glu Asp Leu Leu Ser Pro Pro Gln Asp Thr Ser Thr Gly 870
Leu Glu Glu Val Met Glu Gln Leu Asn Asn Ser Phe Pro Ser Ser 885
Arg Gly Arg Asn Thr Pro Gly Lys Pro Met Arg Glu Asp Thr Met 900

Sequence Number: 11

Length of sequence: 3,163

Form of sequence: nucleic acid

Number of strands: Both morphological form (both)

Topology: straight chain

Kind of sequence: Feature: active - site of cDNA to mRNA arrangement

Arrangement

CGGCCGCTCT AGAGGATCCC CGGGTACCGA GCTCGAATTC CGGAACTCCC GGAGAAAAAC 60

GAATAGGAAA AACTGAAGTG TTACTTTTTT TAAAGCTGCT GAAGTTTGTT GGTTTCTCAT 120

TGTTTTTAAG CCTACTGGAG CAATAAAGTT TGAAGAACTT TTACCAGGTT TTTTTTATCG 180

CTGCCTTGAT ATACACTTTT CAAAATGCTT TGGTGGGAAG AAGTAGAGGA CTGTTATGAA 240

AGAGAAGATG TTCAAAAGAA AACATTCACA AAATGGGTAA ATGCACAATT TTCTAAGTTT 300

GGGAAGCAGC ATATTGAGAA CCTCTTCAGT GACCTACAGG ATGGGAGGCG CCTCCTAGAC 360

CTCCTCGAAG GCCTGACAGG GCAAAAACTG CCAAAAGAAA AAGGATCCAC AAGAGTTCAT 420

GCCCTGAACA ATGTCAACAA GGCACTGCGG GTTTTGCAGA ACAATAATGT TGATTTAGTG 480

AATATTGGAA GTACTGACAT CGTAGATGGA AATCATAAAC TGACTCTTGG TTTGATTTGG 540

AATATAATCC TCCACTGGCA GGTCAAAAAT GTAATGAAAA ATATCATGGC TGGATTGCAA 600

CAAACCAACA GTGAAAAGAT TCTCCTGAGC TGGGTCCGAC AATCAACTCG TAATTATCCA 660

CAGGTTAATG TAATCAACTT CACCACCAGC TGGTCTGATG GCCTGGCTTT GAATGCTCTC 720

ATCCATAGTC ATAGGCCAGA CCTATTTGAC TGGAATAGTG TGGTTTGCCA GCAGTCAGCC 780

ACACAACGAC TGGAACATGC ATTCAACATC GCCAGATATC AATTAGGCAT AGAGAAACTA 840

CTCGATCCTG AAGATGTTGA TACCACCTAT CCAGATAAGA AGTCCATCTT AATGTACATC 900

ACATCACTCT TCCAAGTTTT GCCTCAACAA GTGAGCATTG AAGCCATCCA GGAAGTGGAA 960

GCCCACAGGG ACTTTGGTCC AGCATCTCAG CACTTTCTTT CCACGTCTGT CCAGGGTCCC 1020

TGGGAGAGAG CCATCTCGCC AAACAAAGTG CCCTACTATA TCAACCACGA GACTCAAACA 1080

ACTTGCTGGG ACCATCCCAA AATGACAGAG CTCTACCAGT CTTTAGCTGA CCTGAATAAT 1140

GTCAGATTCT CAGCTTATAG GACTGCCATG AAACTCCGAA GACTGCAGAA GGCCCTTTGC 1200

TTGGATCTCT TGAGCCTGTC AGCTGCATGT GATGCCTTGG ACCAGCACAA CCTCAAGCAA 1260

AATGACCAGC CCATGGATAT CCTGCAGATT ATTAATTGTT TGACCACTAT TTATGACCGC 1320

CTGGAGCAAG AGCACAACAA TTTGGTCAAC GTCCCTCTCT GCGTGGATAT GTGTCTGAAC 1380

TGGCTGCTGA ATGTTTATGA TACGGGACGA ACAGGGAGGA TCCGTGTCCT GTCTTTTAAA 1440

ACTGGCATCA TTTCCCTGTG TAAAGCACAT TTGGAAGACA AGTACAGATA CCTTTTCAAG 1500

CAAGTGGCAA GTTCAACAGG ATTTTGTGAC CAGCGCAGGC TGGGCCTCCT TCTGCATGAT 1560

TCTATCCAAA TTCCAAGACA GTTGGGTGAA GTTGCATCCT TTGGGGGCAG TAACATTGAG 1620

CCAAGTGTCC GGAGCTGCTT CCAATTTGCT AATAATAAGC CAGAGATCGA AGCGGCCCTC 1680

TTCCTAGACT GGATGAGACT GGAACCCCAG TCCATGGTGT GGCTGCCCGT CCTGCACAGA 1740

GTGGCTGCTG CAGAAACTGC CAAGCATCAG GCCAAATGTA ACATCTGCAA AGAGTGTCCA 1800

ATCATTGGAT TCAGGTACAG GAGTCTAAAG CACTTTAATT ATGACATCTG CCAAAGCTGC 1860

TTTTTTTCTG GTCGAGTTGC AAAAGGCCAT AAAATGCACT ATCCCATGGT GGAATATTGC 1920

ACTCCGACTA CATCAGGAGA AGATGTTCGA GACTTTGCCA AGGTACTAAA AAACAAATTT 1980

CGAACCAAAA GGTATTTTGC GAAGCATCCC CGAATGGGCT ACCTGCCAGT GCAGACTGTC 2040

TTAGAGGGGG ACAACATGGA AACTCCCGTT ACTCTGATCA ACTTCTGGCC AGTAGATTCT 2100

GCGCCTGCCT CGTCCCCTCA GCTTTCACAC GATGATACTC ATTCACGCAT TGAACATTAT 2160

GCTAGCAGGC TAGCAGAAAT GGAAAACAGC AATGGATCTT ATCTAAATGA TAGCATCTCT 2220

CCTAATGAGA GCATAGATGA TGAACATTTG TTAATCCAGC ATTACTGCCA AAGTTTGAAC 2280

CAGGACTCCC CCCTGAGCCA GCCTCGTAGT CCTGCCCAGA TCTTGATTTC CTTAGAGAGT 2340

GAGGAAAGAG GGGAGCTAGA GAGAATCCTA GCAGATCTTG AGGAAGAAAA CAGGAATCTG 2400

CAAGCAGAAT ATGACCGTCT AAAGCAGCAG CACGAACATA AAGGCCTGTC CCCACTGCCG 2460

TCCCCTCCTG AAATGATGCC CACCTCTCCC CAGAGTCCCC GGGATGCTGA GCTCATTGCT 2520

GAGGCCAAGC TACTGCGTCA ACACAAAGGC CGCCTGGAAG CCAGGATGCA AATCCTGGAA 2580

GACCACAATA AACAGCTGGA GTCACAGTTA CACAGGCTAA GGCAGCTGCT GGAGCAACCC 2640

CAGGCAGAGG CCAAAGTGAA TGGCACAACG GTGTCCTCTC CTTCTACCTC TCTACAGAGG 2700

TCCGACAGCA GTCAGCCTAT GCTGCTCCGA GTGGTTGGCA GTCAAACTTC GGACTCCATG 2760

GGTGAGGAAG ATCTTCTCAG TCCTCCCCAG GACACAAGCA CAGGGTTAGA GGAGGTGATG 2820

GAGCAACTCA ACAACTCCTT CCCTAGTTCA AGAGGAAGAA ATACCCCTGG AAAGCCAATG 2880

AGAGAGGACA CAATGTAGGA AGTCTTTTCC ACATGGCAGA TGATTTGGGC AGAGCGATGG 2940

AGTCCTTAGT ATCAGTCATG ACAGATGAAG AAGGAGCAGA ATAAATGTTT TACAACTCCT 3000

GATTCCCGCA TGGTTTTTAT AATATTCATA CAACAAAGAG GATTAGACAG TAAGAGTTTA 3060

CAAGAAATAA ATCTATATTT TTGTGAAGGG TAGTGGTATT ATACTGTAGA TTTCAGTAGT 3120

TTCTAAGTCT GTTATTGTTT TGTTGGGGAT CCTCTAGAGT CGA 3163

Sequence Number: 12

Length of sequence: 897

Form of sequence: amino acid

Topology: straight chain

Kind of sequence: protein

Arrangement

Met Leu Trp Trp Glu Glu Val Glu Asp Cys Tyr Glu Arg Glu Asp 15

Val Gln Lys Lys Thr Phe Thr Lys Trp Val Asn Ala Gln Phe Ser 30

Lys Phe Gly Lys Gln His Ile Glu Asn Leu Phe Ser Asp Leu Gln 45

Asp Gly Arg Arg Leu Leu Asp Leu Leu Glu Gly Leu Thr Gly Gln 60

Lys Leu Pro Lys Glu Lys Gly Ser Thr Arg Val His Ala Leu Asn 75

Asn Val Asn Lys Ala Leu Arg Val Leu Gln Asn Asn Asn Val Asp 90

Leu Val Asn Ile Gly Ser Thr Asp Ile Val Asp Gly Asn His Lys 105

Leu Thr Leu Gly Leu Ile Trp Asn Ile Ile Leu His Trp Gln Val 120

Lys Asn Val Met Lys Asn Ile Met Ala Gly Leu Gln Gln Thr Asn 135

Ser Glu Lys Ile Leu Leu Ser Trp Val Arg Gln Ser Thr Arg Asn 150

Tyr Pro Gln Val Asn Val Ile Asn Phe Thr Thr Ser Trp Ser Asp 165

Gly Leu Ala Leu Asn Ala Leu Ile His Ser His Arg Pro Asp Leu 180

Phe Asp Trp Asn Ser Val Val Cys Gln Gln Ser Ala Thr Gln Arg 195

Leu Glu His Ala Phe Asn Ile Ala Arg Tyr Gln Leu Gly Ile Glu 210

Lys Leu Leu Asp Pro Glu Asp Val Asp Thr Thr Tyr Pro Asp Lys 225

Lys Ser Ile Leu Met Tyr Ile Thr Ser Leu Phe Gln Val Leu Pro 240

Gln Gln Val Ser Ile Glu Ala Ile Gln Glu Val Glu Ala His Arg 255

Asp Phe Gly Pro Ala Ser Gln His Phe Leu Ser Thr Ser Val Gln 270

Gly Pro Trp Glu Arg Ala Ile Ser Pro Asn Lys Val Pro Tyr Tyr 285

Ile Asn His Glu Thr Gln Thr Thr Cys Trp Asp His Pro Lys Met 300

Thr Glu Leu Tyr Gln Ser Leu Ala Asp Leu Asn Asn Val Arg Phe 315

Ser Ala Tyr Arg Thr Ala Met Lys Leu Arg Arg Leu Gln Lys Ala 330

Leu Cys Leu Asp Leu Leu Ser Leu Ser Ala Ala Cys Asp Ala Leu 345

Asp Gln His Asn Leu Lys Gln Asn Asp Gln Pro Met Asp Ile Leu 360

Gln Ile Ile Asn Cys Leu Thr Thr Ile Tyr Asp Arg Leu Glu Gln 375

Glu His Asn Asn Leu Val Asn Val Pro Leu Cys Val Asp Met Cys 390

Leu Asn Trp Leu Leu Asn Val Tyr Asp Thr Gly Arg Thr Gly Arg 405

Ile Arg Val Leu Ser Phe Lys Thr Gly Ile Ile Ser Leu Cys Lys 420

Ala His Leu Glu Asp Lys Tyr Arg Tyr Leu Phe Lys Gln Val Ala 435

Ser Ser Thr Gly Phe Cys Asp Gln Arg Arg Leu Gly Leu Leu Leu 450

His Asp Ser Ile Gln Ile Pro Arg Gln Leu Gly Glu Val Ala Ser 465

Phe Gly Gly Ser Asn Ile Glu Pro Ser Val Arg Ser Cys Phe Gln 480

Phe Ala Asn Asn Lys Pro Glu Ile Glu Ala Ala Leu Phe Leu Asp 495

Trp Met Arg Leu Glu Pro Gln Ser Met Val Trp Leu Pro Val Leu 510

His Arg Val Ala Ala Ala Glu Thr Ala Lys His Gln Ala Lys Cys 525

Asn Ile Cys Lys Glu Cys Pro Ile Ile Gly Phe Arg Tyr Arg Ser 540

Leu Lys His Phe Asn Tyr Asp Ile Cys Gln Ser Cys Phe Phe Ser 555

Gly Arg Val Ala Lys Gly His Lys Met His Tyr Pro Met Val Glu 570

Tyr Cys Thr Pro Thr Thr Ser Gly Glu Asp Val Arg Asp Phe Ala 585

Lys Val Leu Lys Asn Lys Phe Arg Thr Lys Arg Tyr Phe Ala Lys 600

His Pro Arg Met Gly Tyr Leu Pro Val Gln Thr Val Leu Glu Gly 615

Asp Asn Met Glu Thr Pro Val Thr Leu Ile Asn Phe Trp Pro Val 630

Asp Ser Ala Pro Ala Ser Ser Pro Gln Leu Ser His Asp Asp Thr 645

His Ser Arg Ile Glu His Tyr Ala Ser Arg Leu Ala Glu Met Glu 660

Asn Ser Asn Gly Ser Tyr Leu Asn Asp Ser Ile Ser Pro Asn Glu 675

Ser Ile Asp Asp Glu His Leu Leu Ile Gln His Tyr Cys Gln Ser 690

Leu Asn Gln Asp Ser Pro Leu Ser Gln Pro Arg Ser Pro Ala Gln 705

Ile Leu Ile Ser Leu Glu Ser Glu Glu Arg Gly Glu Leu Glu Arg 720

Ile Leu Ala Asp Leu Glu Glu Glu Asn Arg Asn Leu Gln Ala Glu 735

Tyr Asp Arg Leu Lys Gln Gln His Glu His Lys Gly Leu Ser Pro 750

Leu Pro Ser Pro Pro Glu Met Met Pro Thr Ser Pro Gln Ser Pro 765

Arg Asp Ala Glu Leu Ile Ala Glu Ala Lys Leu Leu Arg Gln His 780

Lys Gly Arg Leu Glu Ala Arg Met Gln Ile Leu Glu Asp His Asn 795

Lys Gln Leu Glu Ser Gln Leu His Arg Leu Arg Gln Leu Leu Glu 810

Gln Pro Gln Ala Glu Ala Lys Val Asn Gly Thr Thr Val Ser Ser 825

Pro Ser Thr Ser Leu Gln Arg Ser Asp Ser Ser Gln Pro Met Leu 840

Leu Arg Val Val Gly Ser Gln Thr Ser Asp Ser Met Gly Glu Glu 855

Asp Leu Leu Ser Pro Pro Gln Asp Thr Ser Thr Gly Leu Glu Glu 870

Val Met Glu Gln Leu Asn Asn Ser Phe Pro Ser Ser Arg Gly Arg 885

Asn Thr Pro Gly Lys Pro Met Arg Glu Asp Thr Met 897

[Brief Explanation of the Drawing(s)]

[Figure 1]

Figure 1 is something which shows construction of shortening type dystrophin gene which has rod repeat of various numbers.

A of Figure 1 is something which shows human total length type dystrophin gene, mini- dystrophin gene and list of shortening type dystrophin cDNA which is produced newly.

As for B of Figure 1, the ΔDysAX2 (AX2), the ΔDysAX (AX11), the ΔDysAH3

(AH3) and reconstruction in the ΔDysM3 (M3) it is something which shows amino acid sequence of rod repeat which is done.

As for C of Figure 1, the ΔDysH1 (H1) and it is something which shows amino acid sequence of junction region in the ΔDysH4 (H4).

[Figure 2]

Figure 2 is something which shows result of introduction to mouse skeletal muscle cell stocks of shortening type dystrophin cDNA which uses adenoviridae vector.

[Figure 3]

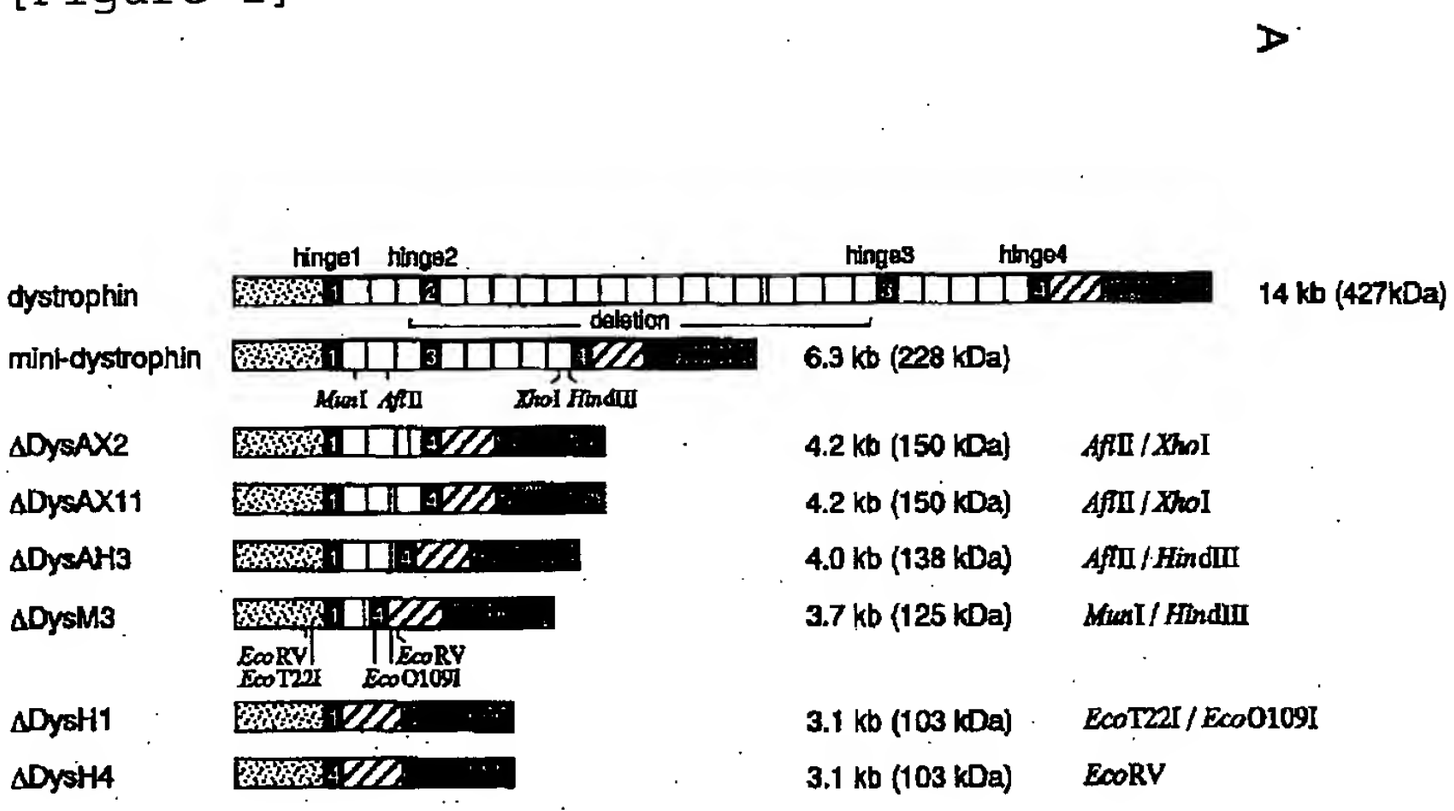
Figure 3 adenoviridae vector is photograph which is substituted to drawing which shows introduction to skeletal muscle of mdx mouse of the shortening type dystrophin cDNA which uses one.

[Figure 4]

As for Figure 4, it is a photograph which is substituted to drawing which shows recovery of dystrophin connection protein in plasmalemma of mdx skeletal muscle which AxCA ΔDysM3 injection is done.

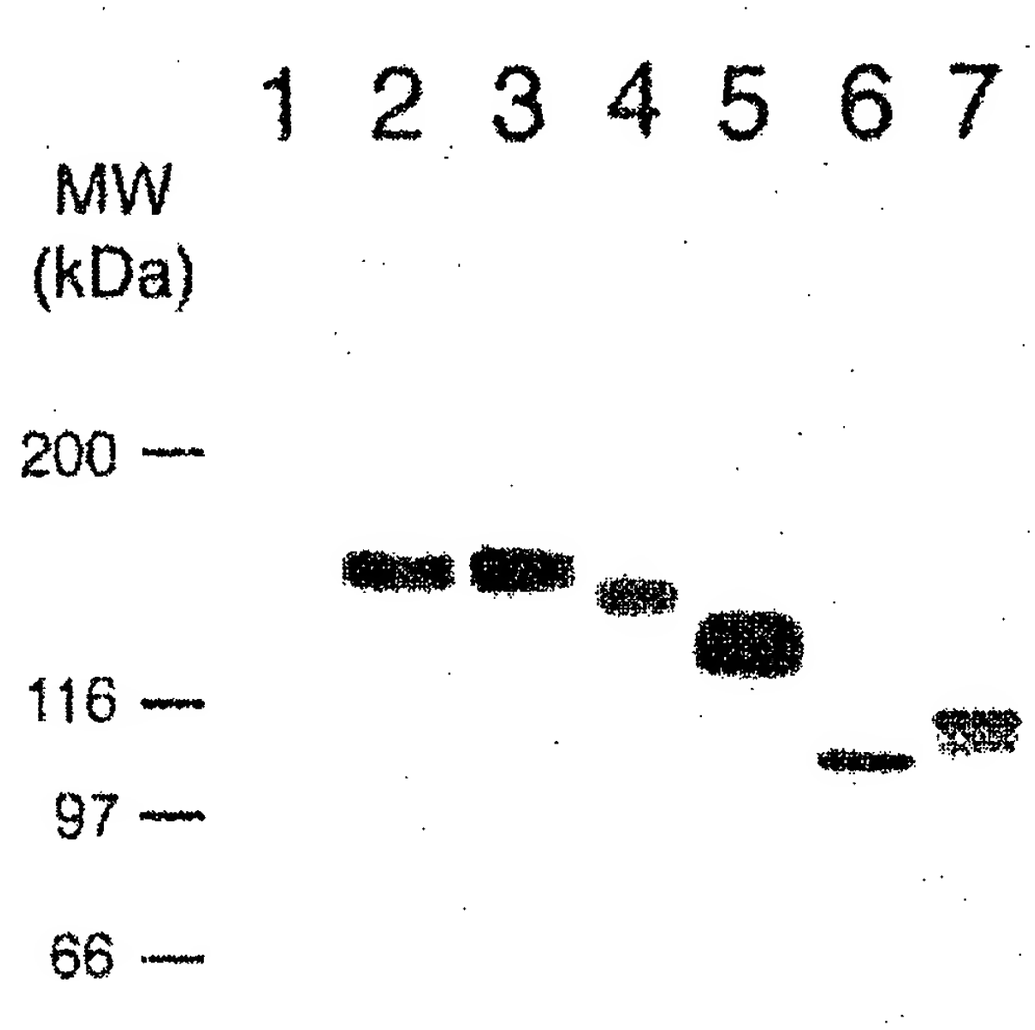
Drawings

[Figure 1]



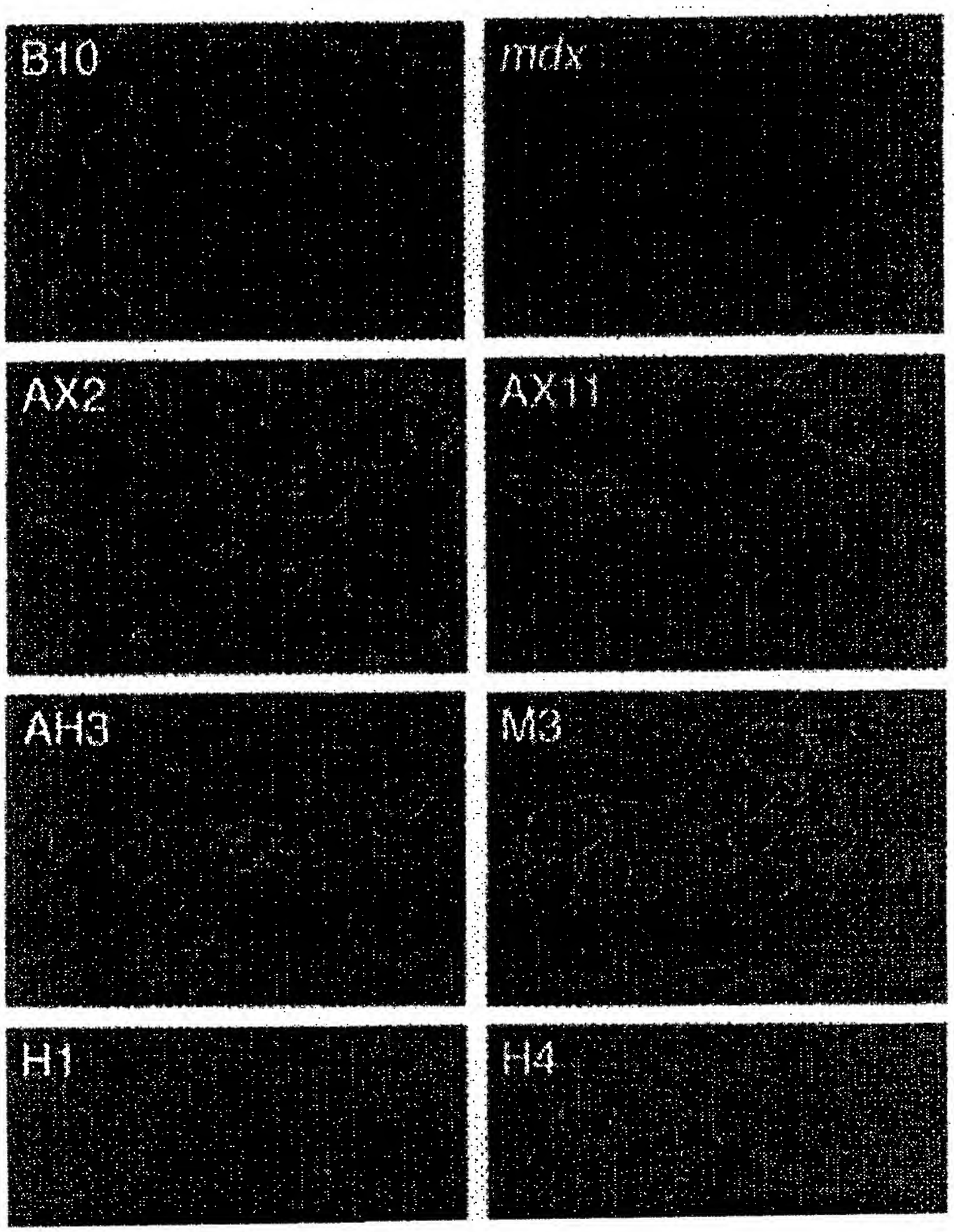
[Figure 2]

図面代用写真



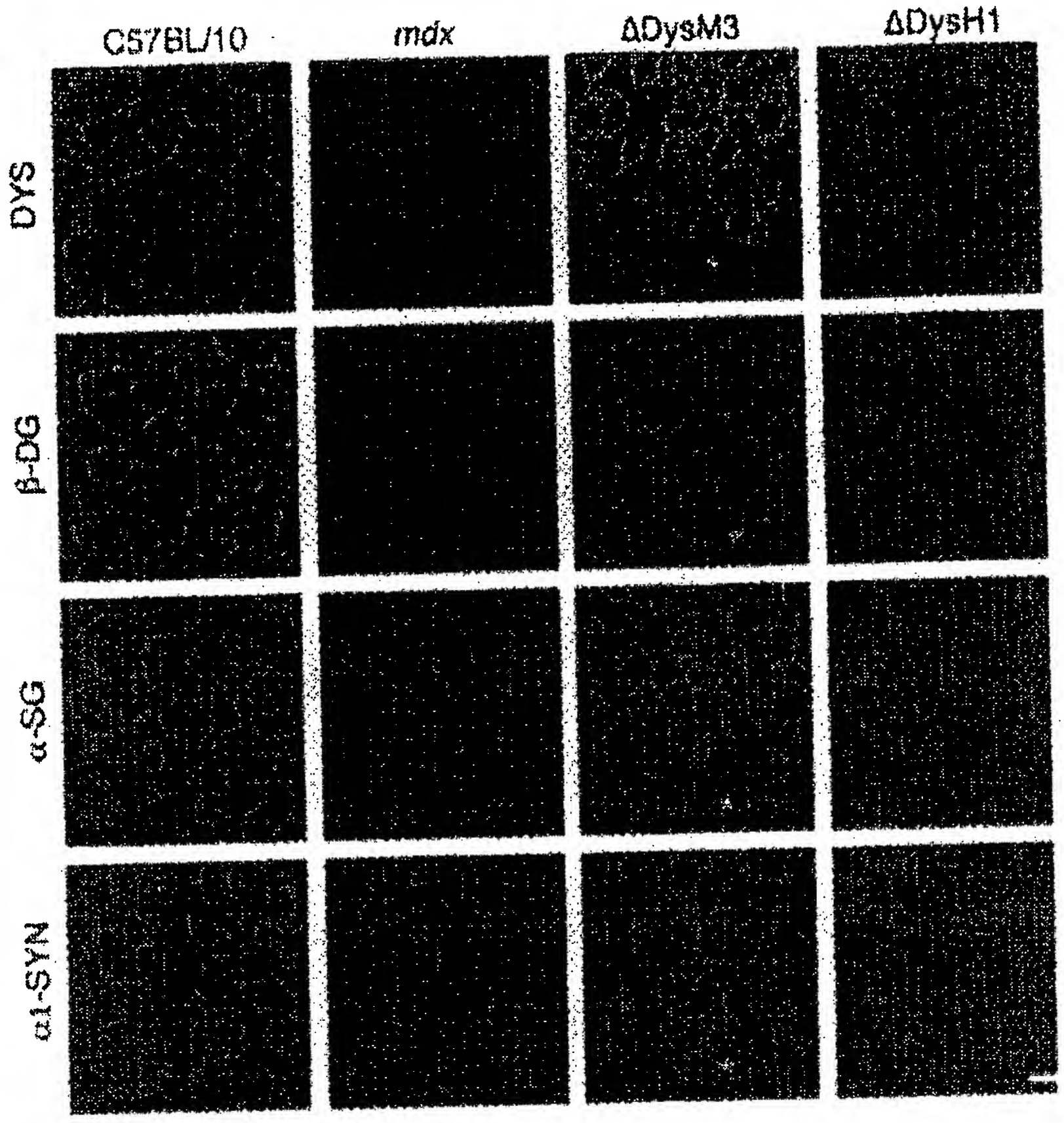
[Figure 3]

図面代用写真



[Figure 4]

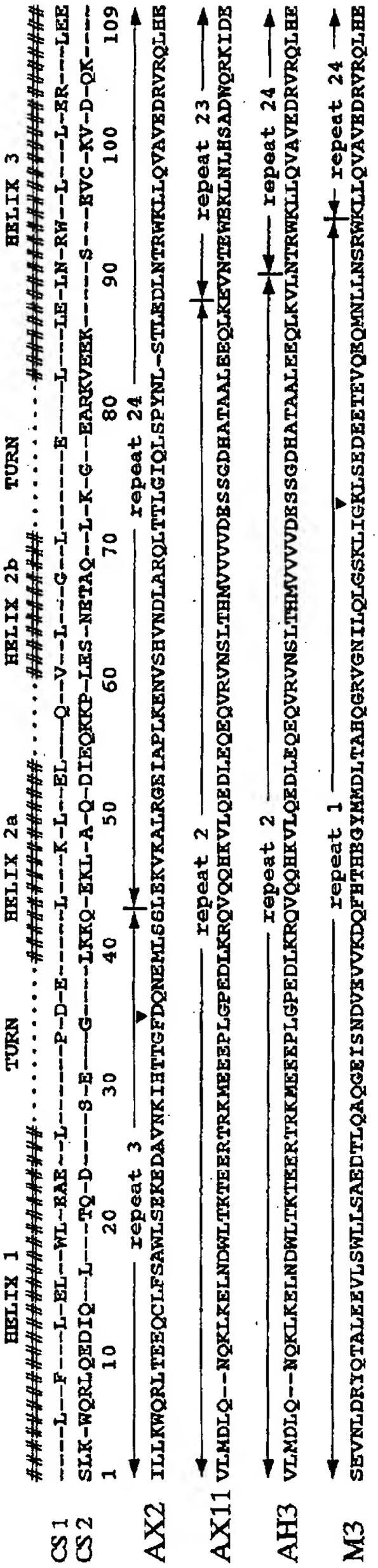
図面代用写真



JP1999318467A

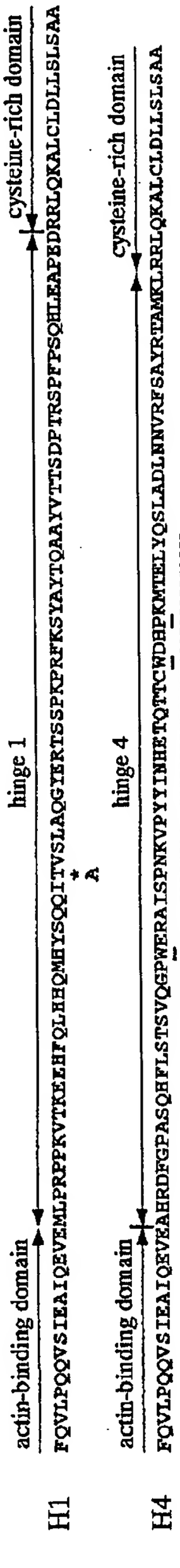
1999-11-24

B


HELIX 1
HELIX 2a
TURN
HELIX 2b
TURN
HELIX 3
CS1
CS2
AX2
AX11
AH3
M3
repeat 1
repeat 2
repeat 3
repeat 23
repeat 24

[Figure 1]

C


cysteine-rich domain
AAYYTTSDPTRSPFPSQHLEAPEDRRLQKALCLDLLSLSAA
hinge 1
ALPRPPKVTKEEHIQLHEQMEYSQQITVSLAQGYERTSSPKPRFKSYAYTQAAYV
A
cysteine-rich domain
VQSLADLMNVRFSAYRTAMKLRRLQKALCLDLLSLSAA
hinge 4
AHRDFGPASQHFLSTSVQGPWERAISPNKVPYYINHETQTTCWDHPKMTELYQSL
actin-binding domain
H1 FQVLPQQVSIEAIQEVEMLPR
actin-binding domain
H4 FQVLPQQVSIEAIQEVEAHRI

[Figure 1]

July 21, 1998

Specification

0019

Modification

{0019} Total length type dystrophin gene, code has done actin binding domain, rod domain, cysteine rich domain, and C terminal domain from N terminal.

These inventors constructed rod shortening type dystrophin cDNA of 6 kinds which furthermore shorten rod domain with human mini- dystrophin gene (6.3 kb) which has 8 rod repeat as material (Figure 1).

All structure has left act in binding domain, cysteine rich domain, and C terminal domain of N terminal.

Specification

0020

Modification

The ΔDysAX2, AX11, AH3 and M3 which {0020} design are done, respectively, have both of rod repeat and hinge 1 and hinge 4 of 3, 3, 2 and 1.

On one hand, as for the ΔDysH1 or H4, as for rod repeat it does not have completely, respectively, hinge 1 has which of 4 (Figure 1, Figure 6).

Base sequence of primer and oligonucleotide which are used for constructing these cDNA is shown in Table 1 of Working Example 1 which it mentions later.

Specification

0023

Modification

{0023} plasmid pBSBMD and primer F1/ R1 which are acquired (Table 1 reference) or after cutting off PCR fragment which amplifying is done, with AflII/ XhoI, it inserted in AflII/ XhoI site of pBSBMD with F2/

R2 (Table 1 reference), respectively, produced the pBSΔ DysAX2 or pBSΔ DysAX11.

Next, after cutting off PCR product which amplifying is done with the MunI/ Hind III, it inserted in MunI/ Hind II Isite of pBSBMD with pBSBMD and the primer F4/ R4 (Table 1 reference) of template, produced pBSΔ DysM3.

Consequently, fragment which is produced with earning ring of oligonucleotide F3/ R3 (Table 1 reference), was used for connection of AflII/ Hind III site of the pBSBMD, pBSΔ DysAH3 was produced.

Occasion where it connects, in order to maintain triple helical structure of the rod repeat, design it did these inserted fragment.

Amino acid sequence of rod repeat which it connects is shown in Figure 5.

Specification

0024

Modification

{0024} As a result, the ΔDysAX2, AX11, AH3 and M3 keep actin binding domain, cysteine rich domain and the C terminal domain of N terminal, furthermore respectively have both of the rod repeat and hinge 1 and 4 of 3, 3, 2 and 1.

It produced the ΔDysH1 and plasmid of 2 it has cDNA of the ΔDysH4, from pBSΔDysM3 (Figure 1).

In order to exclude EcoO109I site of 1, it cut off pBSΔDysM3 with ApaI, after smoothing, self-ligation did, produced pBSΔ DysM3b.

Using pBSΔDysM3 and primer F5/ R5 (Table 1 reference) of template, after cutting off PCR product which amplifying is done with EcoT22I/ EcoO109I, it inserted this in EcoT22I/ EcoO109I site of pBSΔ DysM3b, produced pBSΔ DysH1.

Specification

0025

Modification

For producing {0025} pBSΔ DysH4, pBSΔDysM3 was designatedas template, primer F5/ R6 (Table 1 reference) or F6/ R7 (Table 1 reference) was used and PCR reaction of 2 kinds was done separately.

Using primer F5/ R7 (Table 1 reference) with mixture of PCR product of 2 kinds which it acquires as template, it did PCR reaction of second.

After cutting off fragment which it acquires with EcoRV, this it inserted between EcoRV site of 2 in pBSΔ DysM3.

Amino acid sequence of junction region is shown in Figure 6.

As for the ΔDysH1 or H4 which it acquires, as for rod repeat it does not have completely, respectively, hinge 1 has which of 4 (Figure 1).

Specification

0026

Modification

{0026} Figure 1, Figure 5 and Figure 6 is something which shows construction of the shortening type dystrophin gene which has rod repeat of various numbers.

Figure 1 is human total length type dystrophin gene, mini- dystrophin gene and list figure of shortening type dystrophin cDNA which is produced newly.

The ΔDysAX2, ΔDysAX, ΔDysAH3 and in order to construct the ΔDysM3, it cut off with restriction enzyme which shows rod domain of center of themini-dystrophin cDNA in right side of respective structure.

In order to reconstruct rod repeat structure, using PCR amplifying fragment or synthetic DNA fragment, it connected both ends which it

acquires.

The ΔDysH1 and in order to construct the ΔDysH4, after cutting off, using PCR amplifying fragment with restriction enzyme which illustrates the ΔDysM3, it connected both ends.

Dotted line shows junction.

Size of cDNA and estimated molecular weight of shortening type dystrophin are shown in right side.

Act in binding domain with sporadically box, rod domain with box of the whiteout (Respective repeat is shown with box of 1), cysteine rich domain it illustrates with box where slanted line enters, and C terminal domain with box which attaches shade.

Box of black shows hinge.

As for statement of hinge you followed description of the M. Koenig and L. M. Kunkel.

Specification

0027

Modification

As for {0027} Figure 5, the ΔDysAX2 (AX2), the ΔDysAX11 (AX11), the ΔDysAH3 (AH3) and reconstruction in the ΔDysM3 (M3) amino acid sequence of rod repeat which is done is shown.

Vertical line shows junction rank.

Triangle and dotted line show gap in order alignment of rod repeat optimization to do and position of deficiency, (With M. Koenig and L. M. Kunkel).

CS1 and CS2 show consensus sequence of repeat of 24 of the dystrophin.

As for CS1, amino acid which among Beta vulgaris L. var. saccharifera Alef. (sugar beet) of 24 is found at least in 8 Beta vulgaris L. var. saccharifera Alef. (sugar beet), as for CS2 5, amino acid where is seen 6 or 7 in Beta vulgaris L. var. saccharifera Alef. (sugar beet) is

shown.

Specification

0028

Modification

As for {0028} Figure 6, the ΔDysH1 (H1) and with amino acid sequence ΔDysH1 (H1) of junction region in the ΔDysH4 (H4), you connect directly hinge 1 to the cysteine rich domain.

With the ΔDysH4 (H4), you connect directly act in binding domain to hinge 4.

Tyrosine (T) (star) which is hinge 1 with lineage of XLCM of North America mutation had made in alanine (A).

Dotted line under hinge 4 shows WW domain,; among WW domain, amino acid which most is retained is shown with underline.

Specification

0062

Modification

{0062} Working Example 1 (Construction of rod shortening type dystrophin gene)

Dystrophin gene which furthermore shortens rod domain making use of method which is shown below, 6 kinds was constructed (Figure 1 reference).

First, inserting NotI/ SalI fragment of 6.3 kb which are a human mini-dystrophin [Acsadi, G., Dickson, G., Love, D. R., Jani, A., Walsh, F. S., Gurusinghe, A., Wolff, T. A., and Davies, K. E. (1991) Nature 352, 615 - 818] in NotI/ SalI site of pBluescriptII (SK+) (Stratagene), it produced pBSBMD.

As next, shown plasmid of 4 it has cDNA of shortening type dystrophin (ΔDys) which is named AX2, AX11, AH3, M3 below, it produced.

Base sequence of primer and oligonucleotide which are used for constructing cDNA, is shown in Table

1.

Specification

0065

Modification

{0065} On one hand, it produced the ΔDysH1 and plasmid of 2 it has cDNA of the ΔDysH4, from pBSΔDysM3 (Figure 1 reference).

First, in order one to exclude EcoO109Isite, it cut off the pBSΔDysM3 with ApaI, after smoothing, self ligation did and made pBSΔ DysM3b.

Using pBSΔDysM3 and primer F5/ R5 of template, after cutting off PCR product which amplifying is done, with EcoT22I/EcoO109I, it inserted in the EcoT22I/EcoO109I site of pBSΔ DysM3b, produced pBSΔ DysH1.

For producing pBSΔ DysH4, using primer F5/R6 or F6/ R7, with pBSΔDysM3 as template, it did PCR reaction of 2 kinds, separately.

Using primer F5/ R7 with mixture of PCR product of 2 kinds which it acquires as template, it did PCR reaction of second.

After cutting off fragment which it acquires with EcoRV, this itinserted between EcoRVsite of 2 in pBSΔ DysM3.

Amino acid sequence of junction region is shown in Figure 5 and Figure 6.

Specification

Simple explanation of drawing

Modification

[Brief Explanation of the Drawing(s)]

{Figure 1} Figure 1 is something which shows construction of the shortening type dystrophin gene which has rod repeat of various numbers.

Figure 1 is something which shows human total length type dystrophin

gene, mini- dystrophin gene and list of shortening type dystrophin cDNA which is produced newly.

{Figure 2} Figure 2 is something which shows result of introduction to mouse skeletal muscle cell stocks of shortening type dystrophin cDNA which uses adenoviridae vector.

{Figure 3} Figure 3 is photograph which is substituted to drawing which shows introduction to skeletal muscle of mdx mouse of shortening type dystrophin cDNA which uses adenoviridae vector.

As for {Figure 4} Figure 4, it is a photograph which is substituted to drawing which shows recovery of dystrophin connection protein in plasmalemma of mdx skeletal muscle which AxCA ΔDysM3 injection is done.

As for {Figure 5} Figure 5, the ΔDysAX2 among construction of shortening type dystrophin gene which has rod repeat of various numbers (AX2), the ΔDysAX (AX11), the ΔDysAH3 (AH3) and reconstruction in the ΔDysM3 (M3) it is something which shows amino acid sequence of rod repeat which is done.

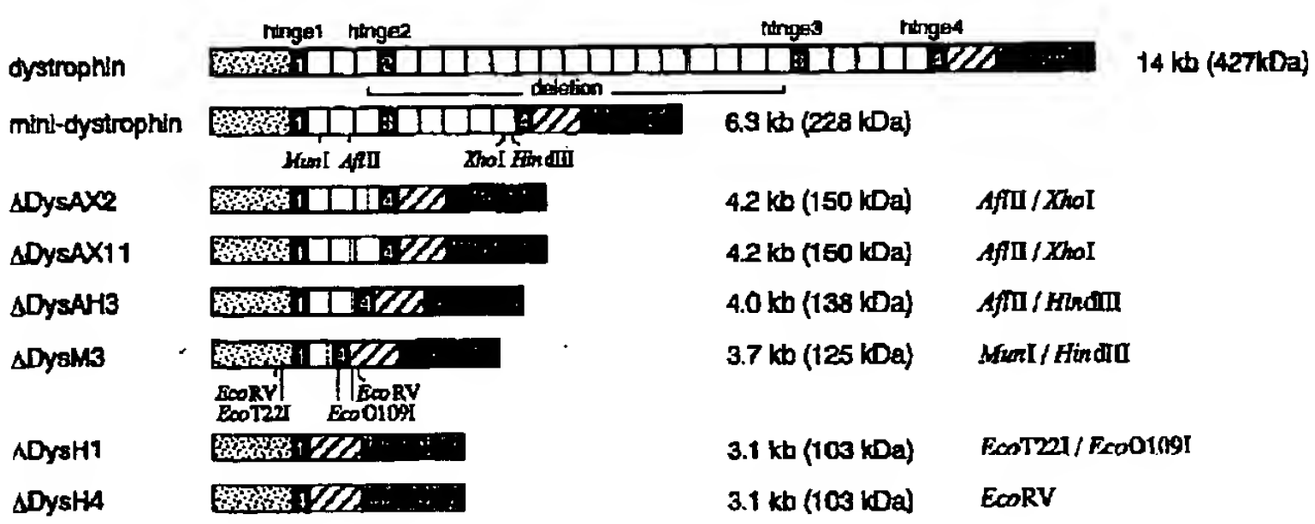
As for {Figure 6} Figure 6, the ΔDysH1 among construction of shortening type dystrophin gene which has rod repeat of various numbers (H1) and it is something which shows amino acid sequence of junction region in the ΔDysH4 (H4).

Drawing

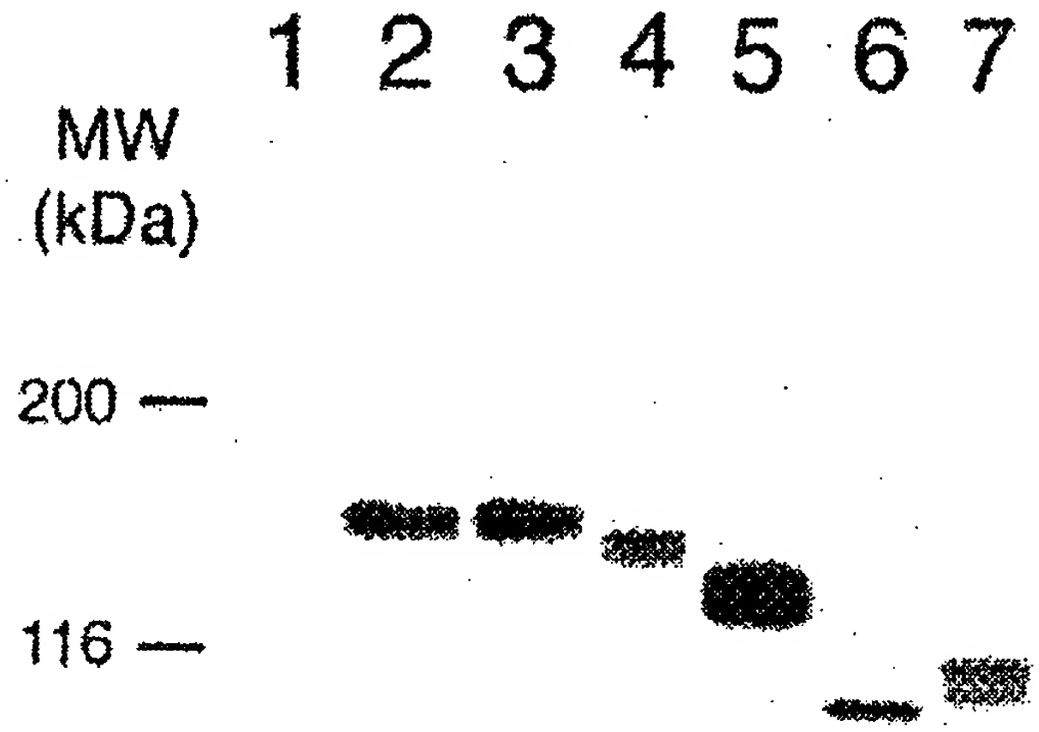
All figure

Modification

[Figure 1]

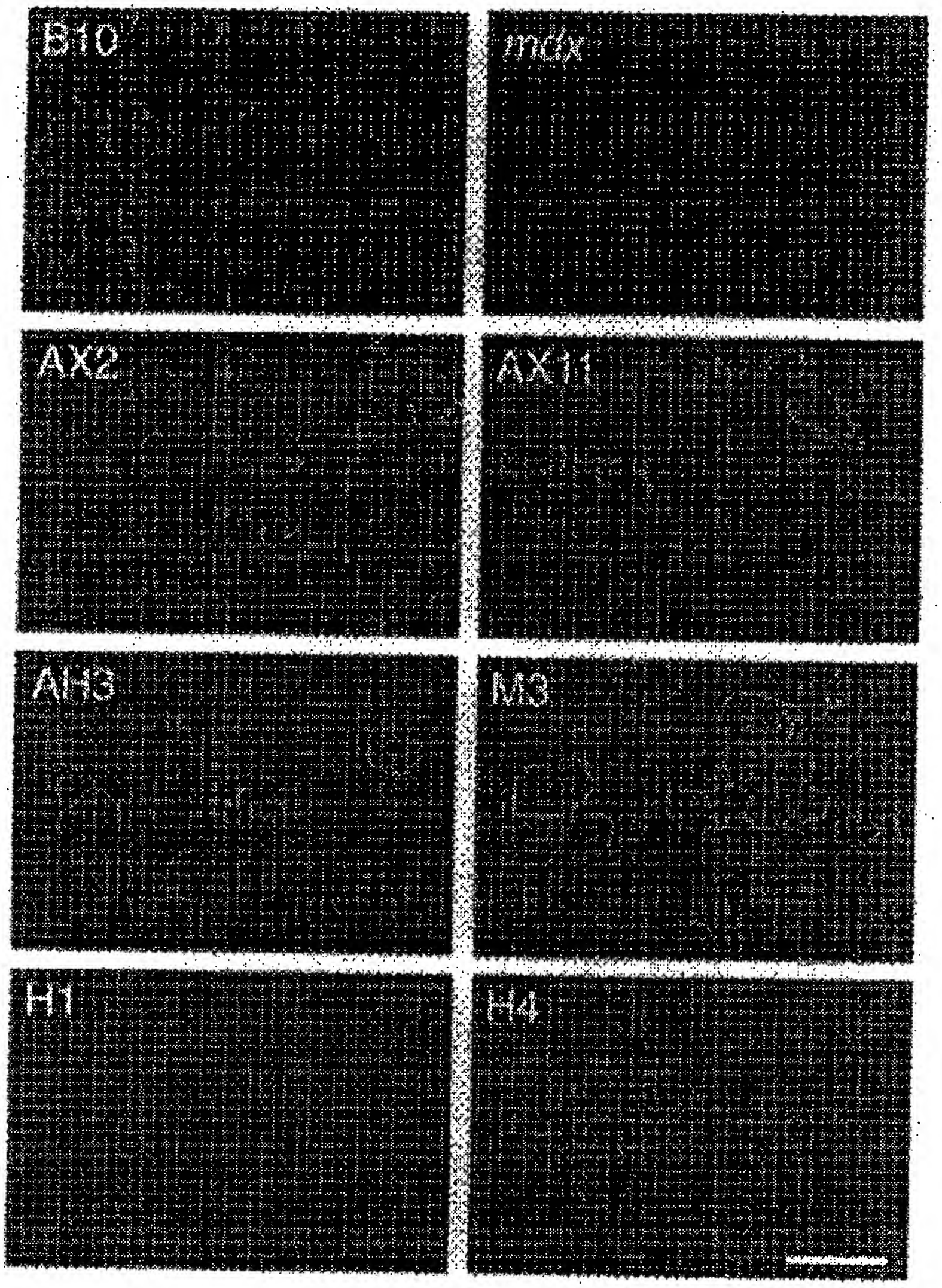


[Figure 2]



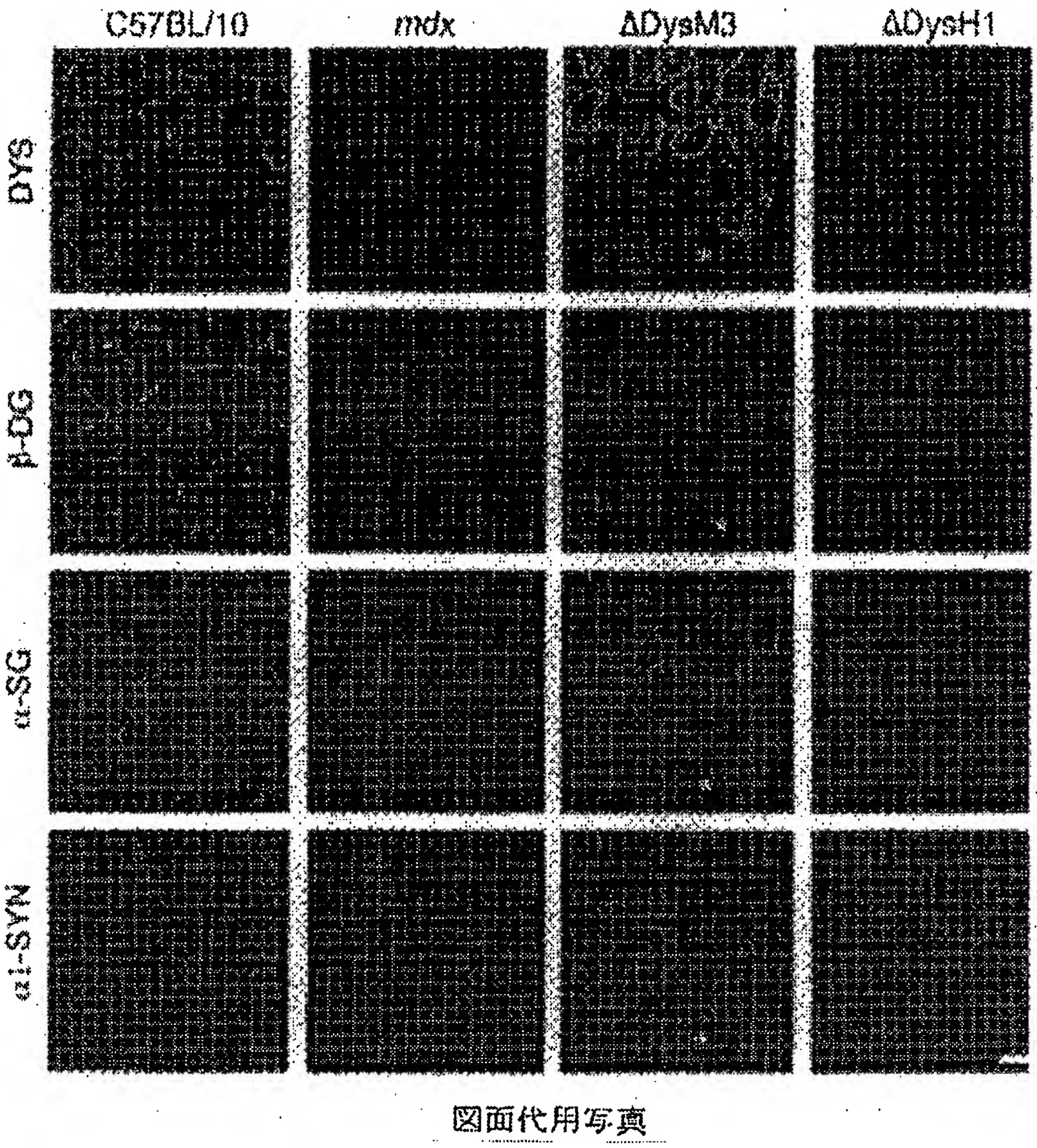
図面代用写真

[Figure 3]



図面代用写真

[Figure 4]



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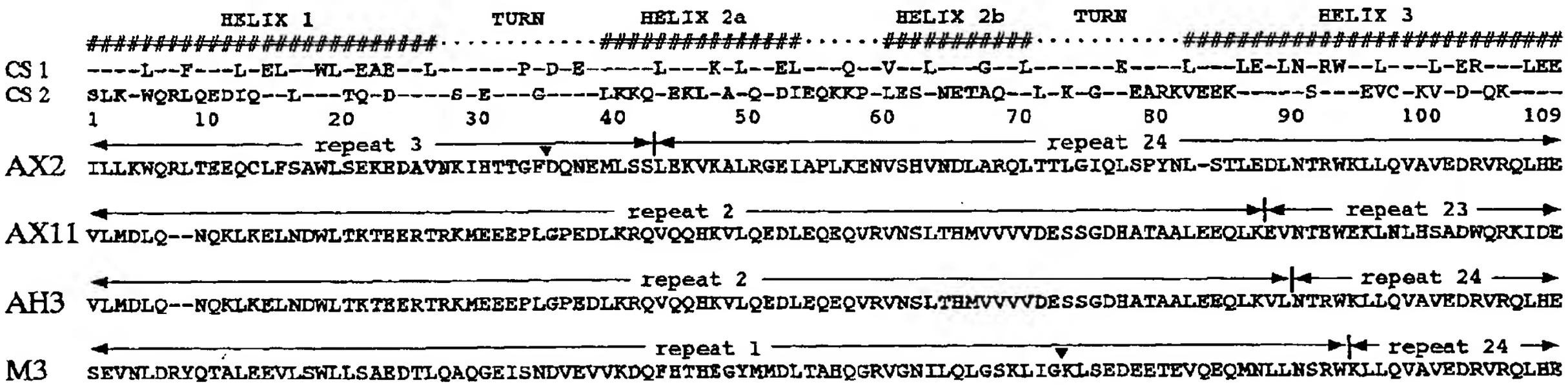
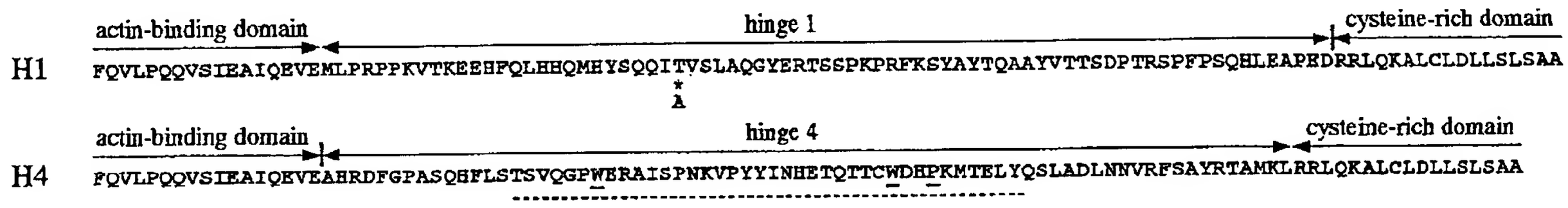
[Figure 5]





[Figure 6]

1999-11-24